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14. ABSTRACT
Purpose: Clinical testing for germline variation in multiple cancer susceptibility genes is available using massively parallel sequencing. Limited information is available for pre-test genetic counseling regarding the spectrum of mutations and variants of uncertain significance (VUSs) in defined patient populations.
Methods: We performed massively parallel sequencing using targeted capture of 22 cancer susceptibility genes in 278 BRCA1/2 negative patients with early onset breast cancer (diagnosed under age 40).
Results: Thirty-one patients (11%) were found to have at least one deleterious or likely deleterious variant. Seven patients (2.5% overall) were found to have deleterious or likely deleterious variants in genes for which clinical guidelines exist for management, namely TP53 (4), CDKN2A (1) MSH2 (1), and MUTYH (double heterozygote). Twenty-four patients (8.6%) had deleterious or likely deleterious variants in a cancer susceptibility gene for which clinical guidelines are lacking, such as CHEK2 and ATM. Fifty-four patients (19%) had at least one VUS, and six patients were heterozygous for a variant in MUTYH.
Conclusion: These data demonstrate that massively parallel sequencing identifies reportable variants in known cancer susceptibility genes in over 30% of patients with early onset breast cancer. However, only rare patients (2.5%) have definitively actionable mutations given current clinical management guidelines.

15. SUBJECT TERMS
early-onset breast cancer, cancer susceptibility, multiplex panel testing, massively parallel sequencing, genetic testing

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INTRODUCTION:

Mutations in the breast and ovarian cancer susceptibility genes, *BRCA1* and *BRCA2*, are found in 10-20% of women with early-onset breast cancer (defined as breast cancer diagnosed under age 40)1. In comparison to women with postmenopausal breast cancer, women with early-onset breast cancer have a worse prognosis with increased recurrence rates, rates of distant metastases, and higher overall mortality. As *BRCA1/2* genetic testing is recommended for all women diagnosed with breast cancer under 40, further expansion of genetic testing to other moderate and high penetrance cancer susceptibility genes is commonly considered for this group. This has the potential to identify women who may benefit from targeted breast cancer screening and prevention strategies aimed at decreasing morbidity and mortality, as has been demonstrated for *BRCA1/2* mutation carriers. However, guidelines do not delineate patient populations for whom testing for mutations in other risk genes is expected to be beneficial, nor how the information of this testing should be applied in clinical management of cancer risk. In addition, rather than focusing on sequential testing of individual, well-studied genes due to defined clinical characteristics of the patient's personal and family histories, these tests which employ massively parallel sequencing technologies via "multiplex panel testing", concurrently screen a large number of genes. A lack of data about the cancer risk and penetrance in women carrying mutations in this expanded panel of genes has made the translation of potential life-saving strategies used in *BRCA1/2* carriers to these women problematic. Whereas frequencies of *BRCA1/2* mutations are well studied, data is needed on the spectrum of variants in the other cancer susceptibility genes in defined patient populations. In Aim 1 of this DOD funded postdoctoral fellowship grant, I therefore set out to study the frequency and type of variants in a panel of cancer susceptibility genes identified by multiplex panel testing in *BRCA1/2* negative women with early-onset breast cancer.

KEYWORDS: early-onset breast cancer, cancer susceptibility, multiplex panel testing, massively parallel sequencing, genetic testing

OVERALL PROJECT SUMMARY:

PROJECT OBJECTIVES:

For additional details and references, please see the Methods section of the manuscript submitted from this work in Appendix 1. Briefly, I identified 280 patients from the cancer genetics research database in the Nathanson Laboratory with the following eligibility criteria: 1) diagnosis of breast cancer under age 40; 2) negative *BRCA1/2* sequencing in a CLIA-approved laboratory; and 3) negative personal or family history of ovarian cancer (***Task 1a1***). For each patient, library preparations for sequencing were made using an Illumina TruSeq Kit and analyzed for quality and quantity using the Agilent DNA 1000 Bioanalyzer (***Task 1a2***). DNA libraries of sufficient quality were pooled pre-capture to 24-plex and hybridized to a custom designed Agilent SureSelect target library covering all coding exons and the flanking 10 base pairs of 28 genes (***Task 1b1***). The genes included 26 study genes plus *BRCA1* and *BRCA2* and were: 1) high penetrance breast cancer susceptibility genes (*CDH1, PTEN, STK11, TP53*); 2) genes known to cause other cancer susceptibility (*CDKN2A, MLH1, MSH2, MSH6, PMS2*); 3) genes known or postulated to be moderate penetrance cancer susceptibility genes (*ATM, BARD1,*

BRIP1, CHEK2, FAM175A,MRE11A, NBN, RAD50, PALB2, RAD51C); 4) *MUTYH*, which leads to autosomal recessive polyposis; and 5) exploratory genes *BABAM1, BRE, BRCC3, MCPH1, PMS1, UIMC1*. Captured libraries were sequenced using paired end sequencing on an Illumina HiSeq at the Next Generation Sequencing Core at the University of Pennsylvania (***Task 1b2***).

Raw sequencing data were aligned to the hg19 assembly of the human genome using Burrows-Wheeler Aligner (BWA) for short-read alignmen. BAM files were processed with Genome Analysis Toolkit (GATK) for detection of single nucleotide variants (SNVs) and insertion/deletion variants (indels) and annotated with ANNOVAR. Data was additionally analyzed using Pindel to improve sensitivity for medium sized indels and xhmm for large genomic rearrangements. Quality control measures were calculated using Picard Tools. Samples were sequenced to a mean coverage of 224X. Two samples were removed from the analysis for having >10% of targets with 0% coverage or <50% of targets with >10X coverage. (***Task1c***)

To identify all single nucleotide variants, small and medium sized insertion/deletions (indels) and large genomic rearrangements, variants were filtered for quality and frequency in population databases (ESP6500 and 1000g) and to remove synonymous missense variants and intronic variants (***Task 1d1***). In order to classify variants into a five-tiered system, a pipeline was developed which integrated posterior probability of pathogenicity data (when available), publically available database calls, protein position of the variant in a functional domain, in silico analysis of effect of variant on conservation with GERP, Siphy and PhyloP and functionality with SIFT, Polyphen2, LRT, MutationTaster and MutationAssessor (***Task 1d2, 1d3***). In order to determine the efficiency and accuracy of our sequencing platform and bioinformatics and variant classification pipeline, we analyzed samples with variants identified by clinical sequencing in *BRCA1, BRCA2, MSH2,* or *PALB2*; these included two nonsense mutations, four indels, two large genomic rearrangements, and 34 single nucleotide variants. 100% of the 42 known variants were identified and correctly classified.

For each identified deleterious and likely deleterious variant in a study sample, a separate stock aliquot of the patient's DNA sample from the aliquot used for massively parallel sequencing was used for Sanger sequencing of the genomic region containing the variant. Primers were developed using NCBI Primer Design software and PCR products were generated with Platinum Taq polymerase.(***Task 1e***).

RESULTS:

For additional details and references, please see the Results section of the manuscript submitted from this work in Appendix 1. Eleven patients carried two *BRCC3*, one *BRE*, five *MCPH1*, three *PMS1* and one *UIMC1* VUSs; no rare variants were identified in *BABAM1*. Given the unclear role of these genes in cancer susceptibility, we excluded these genes from subsequent analysis, restricting the analysis to 22 cancer susceptibility genes.

Characteristics of the early-onset breast cancer study population studied are shown in Table 1 of the manuscript in Appendix 1. Of the 278 patients with high quality sequencing data, 169 (61%) had at least one variant found at <0.1% allele frequency in control public databases. After variant classification, 86 patients (31%) were found to have at least one deleterious variant, likely deleterious variant, or VUS (Figure 1). Thirty-one patients (11%) overall were identified to carry a total of 34 deleterious or likely deleterious variants, 53 patients (19%) had 57 VUSs (including 49 with a VUS only), and six patients (2.2%) were heterozygous for *MUTYH* variants.

Seven patients were identified to have deleterious or likely deleterious variants in a high penetrance cancer susceptibility gene (Figure 1, Figure 2, Table 2 and Results section of the manuscript in Appendix 1). Twenty-four patients were found to have deleterious or likely deleterious variants in genes in which mutations have been associated with a moderate risk of breast cancer (Figure 1, Table 2, Results section of the manuscript in Appendix 1).

The proportion of patients identified to have a clinically reportable variant varied by race, such that 28% of self-reported white patients were found to have at least one reportable variant versus 37% of non-white patients (Figure 1, p=NS and Result section of the manuscript in Appendix 1). The proportion of patients with a deleterious or likely deleterious variant did not vary significantly between white and non-white patients (13% versus 6%, p=NS). The proportion of non-white patients found to carry a VUS was statistically significantly higher than the proportion of white patients, 31% versus 15% (p=0.01). In comparison to deleterious or likely deleterious variant negative patients, there was a statistically significant increase in the rate of second primary malignancies (excluding non-melanoma skin cancers, Table 1 and Results section of the manuscript in Appendix 1, 19% vs 6%, p=0.02) Further results of the correlation between the clinicopathological features of the patient population and variant status is found in the Results section of the manuscript in Appendix 1.

DISCUSSION

Please see the Discussion in the manuscript in Appendix 1.

KEY RESEARCH ACCOMPLISHMENTS:

- This work was presented at a Clinical Sciences Symposium entitled "Next-Generation Sequencing Panels for Cancer Risk Assessment" at the 2014 American Society for Clinical Oncology Annual Meeting. An analysis of my work was presented by Dr. Allison Kurian of Stanford University following the presentation of my abstract.

CONCLUSION:

In year one of this DOD funded postdoctoral fellowship, I have completed all subtasks in Task 1 of the Statement of Work and therefore completed Aim 1, namely to identify the spectrum of variants in 28 known or proposed breast cancer susceptibility genes in over 250 patients with early onset breast cancer using massively parallel sequencing technology (actual total 278 patients) and to confirm interesting variants with traditional Sanger sequencing. I have submitted a manuscript for publication based on this work which reports on the prevalence of clinically reportable variants identified by massively parallel sequencing for 22 known inherited cancer susceptibility genes of the 28 assayed in 278 *BRCA1/2* negative patients with early onset breast cancer (diagnosed under age 40). This is a population of patients for whom *BRCA1/2* testing is recommended and multiplex panel testing by massively parallel sequencing is often clinically considered. My study demonstrates a higher deleterious variant rate of 11% than 7.4-9.5% in other published studies of multiplex panel testing in breast cancer patients, likely reflecting the younger, affected patient population. The rates of variant of uncertain significance (VUSs) vary greatly between studies with other studies reporting as high as an 88% VUS rate; our rate was 19%, likely due our more in-depth variant classification methods. Our patient population contains 24% African Americans, a group not included in other studies, and we demonstrated that although deleterious variant rates were similar between whites and non-whites, VUS rates were higher in non-whites. We found a significnatly higher rate of multiple

primary malignancies in indiviuals with deleterious mutations. Finally, we report that NCCN guidelines are available for management of only 22% of the patients found to have a deleterious variant, or 2.5% of the overall study population. My study therefore provides important data regarding the clinical utility of multiplex panel testing to assist practitioners in weighing the pros and cons when considering clinical panel testing in their patients. I presented this study in a Clinical Sciences Symposium entitled "Next-Generation Sequencing Panels for Cancer Risk Assessment" at the 2014 American Society for Clinical Oncology Annual Meeting (see KEY RESEARCH ACCOMPLISHMENTS). I am now continuing my work in a recently approved change to Aim 2 of this grant (see Appendix 2) to study patients with multiple primary malignancies given the findings in Aim 1. I am currently assaying 50 genes in 176 patients with multiple primary malignancies by massively parallel sequencing. The Variants of Uncertain Significance identified in these two Aims, including 57 VUSs identified in Aim1, will form the basis of the studies for Aim 3 of this grant. Given the variability in VUS classification I have identified and the absence of clinical variant classification guidelines in the literature, I hope to continue my work on developing methods of variant classification, and I have submitted a grant to the American Society of Clinical Oncology (ASCO 2015 Young Investigator Award) for additional funding for this complementary project (see OTHER ACHIEVEMENTS). Finally, the mutations and VUSs identified in this grant are being actively used in two grants funded to Dr. Angela Bradbury at the University of Pennsylvania which is investigating the return of genetic results to patients in the clinic (see OTHER ACHIEVEMENTS).

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

a. Manuscripts

1. Lay Press:

Graff, S. Penn Medicine News, May 14, 2014, "Large Panel Genetic Testing Produces More Questions than Answers in Breast Cancer". (KNM edited and approved, See Appendix 3)

2. Peer-Reviewed Scientific Journals:

Maxwell, K.N., Wubbenhorst, B., Garman, B., D'Andrea, K.P., Rathbun, K., Long, J., Powers, J., Stopfer, J.E., Bradbury, A., Demichele, A., Domchek, S.M., and Nathanson, K.L. (2014) Prevalence of mutations in a panel of breast cancer susceptibility genes in patients with early onset breast cancer. Under review at *Genetics in Medicine.* (See Appendix 1)

Bradbury, A.R., Patrick-Miller, L.,Egleston, B.L., Digiovanni, L., Brower, J., Harris, D., Stevens, E., Maxwell, K.N., Kulkarni, A., Chavez, T., Brandt, A., Long, J., Powers, J., Stopfer, J., Nathanson, K.L., Domchek, S.M. (2014) Uptake and outcomes of multiplex testing for breast cancer susceptibility with a tiered-binned model for informed consent and genetic counseling. Under review at *Journal of Clinical Oncology*

Bradbury, A.R., Patrick-Miller, L., Long, J., Powers, J., Stopfer, J., Forman, A., Rybak, C., Mattie, K., Brandt, A., Chambers, R., Chung, W.K., Churpek, J., Daly, M.B., Digiovanni, L., Faregno-Clark, D., Fetzer, D., Ganschow, P., Grana, G., Gulden, C., Hall, M., Kohler, L.,

Maxwell, K.N., Merrill, S., Montgomery, S., Mueller, R., Nielsen, S., Olopade, O., Rainey, K., Seelaus, C., Nathanson, K.L., Domchek, S.M. (2014) Development of a Tiered and Binned Genetic Counseling Model for Informed Consent in the Era of Multiplex Testing for Cancer Susceptibility. *Genetics in Medicine,* in press.

3. Invited Articles:
Nothing to report.

4. Abstracts:

Maxwell, K.N., Schrader, K., Guidugli, L., Hart, S., Vijai, J., Thomas, T., Wang, W., Wubbenhorst, B., Klein, R., Domchek, S.M., Couch, F.J., Neuhausen, S., Offit, K., Szabo, C., Weitzel, J., Nathanson, K.L. "High and moderate penetrance germline mutations in a number of genes are responsible for a small proportion of familial breast cancer risk in BRCAx families" Poster Presentation, American Association for Cancer Research Annual Meeting, San Diego 2014.

Schrader, K., Maxwell, K.N., Vijai, J., Hart, S., Thomas, T, Wubbenhorst, B., Guidugli, L., Klein, R., Corines, M., Zhang, L., Neuhausen, S., Weitzel, J., Gupta, N., Norton, L., Hudis, C., Getz, G., Daly, M., Lipkin, S., Altshuler, D., Couch, F., Nathanson, K.L., Offit, K. "Determination of cancer susceptibility in probands with breast and ovarian cancer" Poster Presentation, American Association for Cancer Research Annual Meeting, San Diego 2014:

Vijai, J., Hart, S., Thomas, T, Wubbenhorst, B., Guidugli, L., Schrader, K., Maxwell, K.N., Jabobs, L., Villano, D., Klein, R., Lipkin, S., Neuhausen, S., Weitzel, J., Altshuler, D., Couch, F., Offit, K, Nathanson, K.L. "Harmonization of next generation sequencing data within consortia for gene discovery in familial breast cancer" Poster Presentation, American Association for Cancer Research Annual Meeting, San Diego 2014:

b. Presentations:

Clinical Sciences Symposium Oral Presentation, American Society of Clinical Oncology Annual Meeting, Chicago, IL, 2014: Maxwell, K.N., Wubbenhorst, B., Garman, B., D'Andrea, K.P., Rathbun, K., Long, J., Powers, J., Stopfer, J., Bradbury, A.R., DeMichele, A., Domchek, S.M., Nathanson, K.L., "Prevalence of mutations in a panel of breast cancer susceptibility genes in patients with early onset breast cancer"

INVENTIONS, PATENTS AND LICENSES:
Nothing to report.

REPORTABLE OUTCOMES:
Nothing to report.

OTHER ACHIEVEMENTS:

1. Career advancement: I completed my clinical fellowship in Medical Oncology June 30, 2014 during this award period. Due to having this award, I was appointed a Biomedical Postdoctoral Fellow in the Department of Medicine, Division of Hematology-Oncology in order to continue my research.
2. Other projects: My findings from the research results obtained herein are contributing to other projects at the University of Pennsylvania as well. These are: 1) 2013 ACRA in Breast Cancer (PI: Angela Bradbury) "Risks, benefits and utilities of multiplex testing for breast cancer", 2) R01 CA176785-01 (PI: Katherine Nathanson) "Identifying and validating novel susceptibility genes for breast cancer", and 3) R01 funded, pending (PI: Angela Bradbury) "Returning genetic rESearch PanEl results for breast Cancer suscepTibility (The RESPECT Study)". I am a co-author on two Peer-Reviewed Scientific Journal articles due to the first collaboration. I am a first author on one and a co-author on two abstracts due to the second collaboration.
3. Grant applications: One outcome of this first year of research has been the affirmation that classification of genetic variants in the era of massively parallel sequencing for clinical use in oncology is poorly understood and that functional studies of Variants of Uncertain Significance will be critical as per Aim 3 of this DOD funded grant. Therefore, I have applied for additional funding via the American Society of Clinical Oncology Young Investigator Award mechanism to expand the work I am performing with this award. The submitted grant is entitled: "Identification of novel genetic determinants of breast cancer susceptibility in *BRCA1/2* negative patients with familial breast cancer". The grant application has the aim to develop a novel tool to apply American College of Medical Genetics guidelines to classify variants identified by massively parallel sequencing into pathogenicity categories. I then propose to use this tool to classify variants in additional *BRCA1/2* negative familial breast cancer cases, and therefore I will be able to obtain additional VUSs for study in Aim 3 of this DOD funded grant.

REFERENCES:

Please see the References in the manuscript in Appendix 1.

APPENDICES:

Appendix 1: Peer-Reviewed Scientific Journal Article, Maxwell et al, Under review at *Genetics in Medicine*
Appendix 2: Request for Change in Specific Aim 2, submitted and approved August 2014
Appendix 3: News Release from "Penn Medicine News", May 14, 2014: "Large Panel Genetic Testing Produces More Questions than Answers in Breast Cancer"

TRAINING OR FELLOWSHIP AWARD TRAINING GOALS:

Meetings: I attended the 2014 AACR Annual Meeting in April, 2014 in San Diego, California and the 2014 ASCO Annual Meeting in June 2014 in Chicago Illinois. I presented a poster at the AACR meeting and was given the unique opportunity to present the work in this award at a Clinical Sciences Symposium entitled "Next-Generation Sequencing Panels for Cancer Risk Assessment".

Clinical Activities: During the period, I participated in a half-day clinic per week with Dr. Susan

Domchek in Breast Cancer Genetics (9/30/2013 – 3/30/2014) and Dr. Anil Rustgi in GI Cancer Genetics (4/1/2014-6/30/2014). Starting in my Postdoctoral Fellowship as of 7/1/2014 I do not have clinical responsibilities. I am therefore attending three clinical conferences: Mariann and Robert MacDonald Women's Cancer Risk Evaluation Center Clinical Cancer Genetics Conference (presentation of hereditary cancer cases for discussion of clinical management), Rena Rowen Breast Center Breast Cancer Tumor Board (presentation of breast cancer cases for discussion of clinical management) and Gastrointestinal Cancer Tumor Board (presentation of gastrointestinal cancer cases for discussion of clinical management).

Mentoring: I am currently mentoring two undergraduate students in the laboratory, Vishal Patel (University of Pennsylvania ’16) and Abha Kulkarni (University of Pennsylvania ’17).

Research Meetings: I attend and actively present my research at the Nathanson Laboratory Meeting and the Multidisciplinary Sequencing Meeting led by members of the Nathanson Laboratory.

Course work: I intended to apply for the AACR Translational Research for Basic Scientists course in Boston Massachusetts which is occurring Oct. 26-31, 2014. Unfortunately, my father has recently fallen ill and I am unable to be out of town for this period of time. I have therefore been expanding my education in Translational Research by presenting one to three clinical cases at the University of Pennsylvania Perelman School of Medicine Molecular Tumor Board. At this conference, genetic sequencing studies obtained clinically from tumors of patients are presented and participants discuss both clinical care and potential translational research projects emanating from the results. I am specifically in charge of presenting results of a project funded by the University of Pennsylvania 2-PREVENT Translational Center of Excellence in Breast Cancer entitled “METAMORPH” (PI: Dr. Angela DeMichele, Dr. Lewis Chodosh), which is performing targeted sequencing of cancer related genes in metastatic lesions from breast cancer patients. Through presentation of these cases and attendance at Dr. DeMichele’s project meetings, I am learning a significant amount about translational research. I also participate in the monthly conferences of the Basser Research Center for BRCA at which principal investigators performing basic, translational and clinical research relevant to *BRCA1/2* present their research findings.

Genetics in Medicine

Prevalence of mutations in a panel of breast cancer susceptibility genes in BRCA1/2 negative patients with early onset breast cancer

--Manuscript Draft--

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Prevalence of mutations in a panel of breast cancer susceptibility genes in *BRCA1/2* negative patients with early onset breast cancer

Running title: *Panel testing in patients with early onset breast cancer*

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Abstract

Purpose: Clinical testing for germline variation in multiple cancer susceptibility genes is available using massively parallel sequencing. Limited information is available for pre-test genetic counseling regarding the spectrum of mutations and variants of uncertain significance (VUSs) in defined patient populations.

Methods: We performed massively parallel sequencing using targeted capture of 22 cancer susceptibility genes in 278 *BRCA1/2* negative patients with early onset breast cancer (diagnosed under age 40).

Results: Thirty-one patients (11%) were found to have at least one deleterious or likely deleterious variant. Seven patients (2.5% overall) were found to have deleterious or likely deleterious variants in genes for which clinical guidelines exist for management, namely *TP53* (4), *CDKN2A* (1) *MSH2* (1), and *MUTYH* (double heterozygote). Twenty-four patients (8.6%) had deleterious or likely deleterious variants in a cancer susceptibility gene for which clinical guidelines are lacking, such as *CHEK2* and *ATM.* Fifty-four patients (19%) had at least one VUS, and six patients were heterozygous for a variant in *MUTYH*.

Conclusion: These data demonstrate that massively parallel sequencing identifies reportable variants in known cancer susceptibility genes in over 30% of patients with early onset breast cancer. However, only rare patients (2.5%) have definitively actionable mutations given current clinical management guidelines.

Keywords: early-onset breast cancer, cancer susceptibility, multiplex panel testing, massively parallel sequencing, genetic testing

INTRODUCTION

Mutations in the breast and ovarian cancer susceptibility genes, *BRCA1* and *BRCA2,* are found in 10-20% of women with early-onset breast cancer (defined as breast cancer diagnosed under age 40)[1]. In comparison to women with postmenopausal breast cancer, women with early-onset breast cancer have a worse prognosis with increased recurrence rates, rates of distant metastases, and higher overall mortality[2,3]. As *BRCA1/2* genetic testing is recommended for all women diagnosed with breast cancer under 40[4], further expansion of genetic testing to other moderate and high penetrance genes is commonly considered for this group. Further, it has the potential to identify women who may benefit from targeted breast cancer screening and prevention strategies aimed at decreasing morbidity and mortality, as has been demonstrated for *BRCA1*/2 mutation carriers[5,6].

Beyond *BRCA1*/*2*, rare highly penetrant mutations in the genes *CDH1*, *PTEN*, *STK11*, and *TP53* [7-10] lead to cancer susceptibility syndromes, for which the National Cancer Care Network (NCCN) provides guidelines for genetic testing and clinical management[4]. In addition to these high risk genes, breast cancer susceptibility is associated with rare moderate penetrance mutations in an increasing number of genes, such as *ATM*, *CHEK2*, and *PALB2*[11-13], which confer an approximately two to five fold increased risk of breast cancer. Guidelines do not delineate patient populations for whom testing for mutations in moderate risk genes is expected to be beneficial, nor how the information of this testing should be applied in clinical management of cancer risk.

Despite these limitations, clinical testing based on massively parallel sequencing (MPS) is now commercially available for many known or proposed cancer susceptibility genes[14,15]. Rather than focusing on sequential testing of individual, well-studied genes due to defined clinical characteristics of the patient's personal and family histories, these tests concurrently screen a large number of genes. A lack of data about the cancer risk and penetrance in women carrying these mutations has made the translation of potential life-saving strategies used in

BRCA1/2 carriers to these women problematic[16-18]. Whereas frequencies of *BRCA1/2* mutations are well studied, data is needed on the spectrum of variants in the other cancer susceptibility genes in defined patient populations. We report, herein, data on the frequency and type of variants in a panel of cancer susceptibility genes in *BRCA1/2* negative women with early-onset breast cancer.

MATERIALS AND METHODS

Patient ascertainment

The study population was ascertained from academic and community hospital sites within the Penn Cancer Network and The Karmanos Cancer Institute at Wayne State University[19]. The majority of the patient population (253 patients, 91%) was ascertained via the Penn Cancer Network sixteen patients (6%) were from the Karmanos Cancer Institute at Wayne State University, and ascertainment data was not available for nine patients (3%). Acquisition of the patient samples was approved by the Institutional Review Boards of the corresponding institutions, and informed consent was obtained from each participant for use of their samples in genetic studies. Eligibility criteria for the study were: 1) diagnosis of breast cancer under age 40; 2) negative *BRCA1/2* sequencing in a CLIA-approved laboratory; and 3) negative personal or family history of ovarian cancer. Analysis for *BRCA1/2* large genomic rearrangements was not required, although negative clinical testing was available for 28% of patients.

DNA library preparation and sequencing

For each patient, one microgram of constitutional DNA was blunt ended and ligated with adaptors-embedded indexes. DNA quality, fragment size and concentration were measured with an Agilent 2100 Bioanalyzer. DNA libraries of sufficient quality were pooled pre-capture to 24-plex and hybridized to a custom designed Agilent SureSelect target library covering all coding exons and the flanking 10 base pairs of 22 genes. The genes included 20 study genes plus

BRCA1 and *BRCA2* and were: 1) high penetrance breast cancer susceptibility genes (*CDH1, PTEN*, *STK11, TP53*); 2) genes known to cause other cancer susceptibility (*CDKN2A, MLH1, MSH2*, *MSH6, PMS2*); 3) genes known or postulated to be moderate penetrance cancer susceptibility genes (*ATM*, *BARD1*, *BRIP1*, *CHEK2, FAM175A,MRE11A, NBN, RAD50, PALB2, RAD51C*); and 4) *MUTYH*, which leads to autosomal recessive polyposis.

Massively parallel sequencing data analysis

Raw sequencing data were aligned to the hg19 assembly of the human genome using Burrows-Wheeler Aligner (BWA) for short-read alignment (http://sourceforge.net/projects/bio-bwa/files/)[20]. BAM files were processed with Genome Analysis Toolkit (GATK) for detection of single nucleotide variants (SNVs) and insertion/deletion variants (indels) (http://www.broadinstitute.org/gatk/download)[21] and annotated with ANNOVAR (http://www.openbioinformatics.org/annovar/annovar_download.html)[22]. Data was additionally analyzed using Pindel to improve sensitivity for medium sized indels (http://gmt.genome.wustl.edu/pindel/0.2.4/install.html)[23] and xhmm for large genomic rearrangements (https://atgu.mgh.harvard.edu/xhmm/download.shtml)[24]. Quality control measures were calculated using Picard Tools (http://sourceforge.net/projects/picard/files/). Samples were sequenced to a mean coverage of 224X. Three samples were removed from the analysis for having >10% of targets with 0% coverage or <50% of targets with >10X coverage.

To identify all single nucleotide variants, small and medium sized insertion/deletions (indels) and large genomic rearrangements, variants were filtered to remove synonymous missense variants and intronic variants. Variants were removed from analysis if the alternate allele frequency was less than 0.2 and the total number of reads less than 20. All other insertion, deletions, nonsense variants, and splicing variants were retained for further analysis. Variants were kept for further analysis if found at an allele frequency of less than 0.1% in both the ESP6500 (http://evs.gs.washington.edu/EVS/) and 1000G

(http://www.1000genomes.org/data)[25] databases. Variants were analyzed if found at 0.1-1% allele frequency and previously reported to be a breast cancer susceptibility variant. Splicing variants were analyzed with Skippy and PupaSuite[26,27]. All variants were visually inspected in the Integrative Genomics Viewer (IGV, http://www.broadinstitute.org/software/igv/log-in)[28].

Variant classification

In order to classify variants into a five-tiered system, a pipeline was developed which integrated posterior probability of pathogenicity data (when available), publically available database calls, protein position of the variant in a functional domain, in silico analysis of effect of variant on conservation with GERP[29], Siphy[30] and PhyloP[31] and functionality with SIFT[32], Polyphen2[33], LRT[34], MutationTaster[35] and MutationAssessor[36] (Supplementary Table 1). Specifically, variants were first assigned as a Variant of Uncertain Significance (VUS) if a) the posterior probability of pathogenicity > 0.0518 as recorded in the gene's locus specific database (LSDB) if available or b) if the variant was not found in EVS6500, 1000 Genomes and dbSNP databases, if a LSDB was not available. If these conditions were not met, the variant was assigned as a likely benign Variant (i.e. if a) the posterior probability of pathogenicity < 0.0518 as recorded in the LSDB if available or b) if the variant was found in EVS6500, 1000 genomes or dbSNP databases, if a LSDB was not available). Exceptions were made for known pathogenic variants found in EVS6500, 1000G and dbSNP (i.e. *CHEK2* c.1100delC). For the VUSs, variants were upgraded to deleterious variant if called pathogenic by two or more databases (HGMD http://www.hgmd.org/, Clinvar https://www.ncbi.nlm.nih.gov/clinvar/, and the LSDB of the gene (http://www.hgvs.org/dblist/glsdb.html). VUSs were upgraded to likely deleterious variants if at least four of the following five features ("D points") indicated pathogenicity of the variant: 1) position of variant in a biologically important functional domain of the protein known to harbor pathogenic mutations; 2) pathogenic call in one database (HGMD, Clinvar, and the LSDB of the gene); 3) a normalized conservation score (NCS) of >2 (maximum

3); 4) a normalized functional score (NFS) of >4 (maximum 5); and 5) reported non-functional in a published in vitro assay. The Normalized Conservation Score was calculated by NCS = (GERPScore/x) + (PhyloPScore/x) + (SiPhyScore/x), where x=maximum score for each caller in the dataset. The Normalized Functional Score was calculated by NFS =(1-SIFTScore) + PP2HDIVScore + (1-LRTScore) + MutTasterScore + (MutAssessorScore/x), where x=maximum score for each caller in the dataset. If the NFS was between 3-4, the variant was given one D point if the AlignGVGD score (http://agvgd.iarc.fr/agvgd_input.php)[37] was C55 or C65 or if the CONDEL score (http://agvgd.iarc.fr/agvgd_input.php)[38] was "D". For the likely benign variants, these variants were upgraded to VUSs if at least two features ("D points", listed above) indicated pathogenicity of the variant. Likely benign variants were downgraded to benign variants if called a SNP by more than two databases (HGMD, Clinvar, dbSNP and the LSDB of the gene).

Validation of pipeline

In order to determine the efficiency and accuracy of our sequencing platform and bioinformatics and variant classification pipeline, we analyzed samples with variants identified by clinical sequencing in *BRCA1*, *BRCA2*, *MSH2*, or *PALB2*; these included two nonsense mutations, four indels, two large genomic rearrangements, and 34 single nucleotide variants. 100% of the 42 known variants were identified and correctly classified. For each identified deleterious and likely deleterious variant in a study sample, a separate stock aliquot of the patient's DNA sample from the aliquot used for MPS was used for Sanger sequencing of the genomic region containing the variant. Primers were developed using NCBI Primer Design software and PCR products were generated with Platinum Taq polymerase.

Statistical analysis of clinicopathogical variables

Statistical comparisons were made regarding the frequency of patients with certain clinical or pathological features within groups of patients as determined by variant status using a two-tailed Fisher's exact test. Statistical comparisons of age, Penn II scores, and BOADICEA scores between groups of patients depending on variant status was performed using a two-tailed, type 2, Student's t-test. Comparisons were run for deleterious/likely deleterious variant positive versus deleterious/likely deleterious variant negative (including the VUS positive patients in the latter group) and deleterious/likely deleterious variant positive versus deleterious/likely deleterious variant and VUS negative (excluding the VUS positive patients from both groups).

RESULTS

Characteristics of the early-onset breast cancer study population studied are shown in Table 1. Of the 278 patients, 169 (61%) had at least one variant found at <0.1% allele frequency in control public databases. After variant classification, 86 patients (31%) were found to have at least one deleterious variant, likely deleterious variant, or VUS (Figure 1). Thirty-one patients (11%) overall were identified to carry a total of 34 deleterious or likely deleterious variants, 53 patients (19%) had 57 VUSs (including 49 with a VUS only), and six patients (2.2%) were heterozygous for *MUTYH* variants.

Seven patients were identified to have deleterious or likely deleterious variants in a high penetrance cancer susceptibility gene (Figure 1, Table 2). Two patients were found to carry a known pathogenic *TP53* mutation (Figure 2a,b). Two patients, including one African American, were found to carry likely deleterious variants in *TP53*. One patient was identified to have a large genomic rearrangement deleting exon 5 of *MSH2* leading to an in-frame deletion of 65 amino acids of the DNA interacting domain of MSH2. A patient with a history of both early-onset breast cancer and sarcoma was found to carry a known pathogenic missense mutation in *CDKN2A*. Finally, one patient, with a personal history of early onset colon cancer and two

primary breast cancers, was found to be a compound heterozygote for a known pathogenic mutation and a likely deleterious variant in *MUTYH*.

Twenty-four patients were found to have deleterious or likely deleterious variants in genes in which mutations have been associated with a moderate risk of breast cancer. The majority of deleterious or likely deleterious variants in moderate penetrance genes were found in *ATM* and *CHEK2* (Figure 1, Table 2). Single deleterious or likely deleterious variants were found in *ATM* in seven patients and in *CHEK2* in nine patients. One patient was found to carry deleterious variants in both *ATM* and *CHEK2*; of note both variants also were found in her brother with early onset prostate cancer (Figure 2c). In addition, one patient was found to carry two likely deleterious variants *in trans* in *CHEK2*. The remaining six patients had deleterious variants in *MRE11A* (2), *BARD1* (1), *BRIP1* (1), *NBN* (1), and *RAD50* (1). Twenty-seven patients carried a VUS in a high penetrance cancer susceptibility gene, and three of those patients also had a deleterious or likely deleterious variant. Nine patients were found to have a single VUS in *BRCA1* or *BRCA2*, three patients in *TP53* and 12 patients in *MLH1*, *MSH2*, *MSH6*, or *PMS2*; no VUSs were found in *CDH1*, *CDKN2A*, *STK11* or *PTEN*. Three additional patients each carried two VUSs in a high penetrance cancer susceptibility gene. Twenty-six patients were found to have VUSs in moderate penetrance cancer susceptibility genes, *ATM*, *BRIP1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD50*, and *RAD51C*; no VUSs were found in *BARD1*. Finally, six patients carried a single deleterious variant or VUS in *MUTYH* (Figure 1). Three patients were heterozygous for the same known pathogenic *MUTYH* mutation and three were heterozygous for VUSs in *MUTYH*.

The proportion of patients identified to have a clinically reportable variant varied by race, such that 28% of self-reported white patients were found to have at least one reportable variant versus 37% of non-white patients (Figure 1, p=NS). The proportion of patients with a deleterious or likely deleterious variant did not vary significantly between white and non-white patients (13% versus 6%, p=NS). The proportion of non-white patients found to carry a VUS was statistically

significantly higher than the proportion of white patients, 31% versus 15% (p=0.01). Of the 66 African Americans, 7.5% carried a deleterious or likely deleterious variant, which was not statistically significantly different than the proportion of white patients. Of the 27 Ashkenazi Jewish individuals, 22% were found to have a deleterious or likely deleterious variant, compared with 10% of the 234 non-Ashkenazi Jewish individuals (p=NS).

In comparison to deleterious or likely deleterious variant negative patients, there was a statistically significant increase in the rate of second primary malignancies (excluding non-melanoma skin cancers, Table 1, 19% vs 6%, p=0.02) in the deleterious or likely deleterious variant positive patients. In addition, there was a trend towards a higher rate of a bilineal family history of breast cancer in deleterious or likely deleterious variant positive versus negative patients (23% vs 11%, p=0.08). The Penn II *BRCA1/2* prior probability score [39] was statistically significantly higher (27% vs 19%, p=0.04) in deleterious or likely deleterious variant positive patients versus variant negative individuals, as was the BOADICEA [40] score (29% vs 14%, p=0.005).

Only three of the 22 patients with deleterious or likely deleterious variants had ER-invasive breast cancer (Table 1, 14%), one had triple negative breast cancer (*BARD1* p.S551X) and two had ER- Her2+ breast cancer (*TP53* p.P151T and *CHEK2* c.444+1A>G). In contrast, 33% of the patients with no deleterious or likely deleterious variant (+/- a VUS) had ER- invasive breast cancer (p=0.09). Seven of the 20 patients (35%) with a deleterious or likely deleterious variant had Her2+ breast cancer versus 26% of the patients with no deleterious or likely deleterious variant (+/- a VUS, p=NS). Finally, deleterious or likely deleterious variants were found in 13% of the patients with DCIS, 11% of the 116 patients with node positive invasive cancer, and 11% of the 130 patients with node negative invasive breast cancer. The stage distribution was similar between deleterious or likely deleterious variant positive versus negative patients.

DISCUSSION

Using massively parallel sequencing for 22 genes previously associated with cancer susceptibility, we found that 31% of *BRCA1/2* negative patients with early-onset breast cancer and no family history of ovarian cancer have a clinically reportable variant, of which one-third were deleterious or likely deleterious variants. However, clinical guidelines exist for the management of cancer risk in only 2.5% of the patients, those found to have deleterious or likely deleterious variants in *TP53*, *CDKN2A*, *MSH2,* and the *MUTYH* double heterozygote. Currently, there are no standard of care clinical guidelines for the management of cancer risk in the 10% of women with single mutations in a moderate penetrance cancer susceptibility gene and *MUTYH*. Even greater clinical uncertainty exists for the 19% of patients who were found to carry VUSs.

Multiplex panel MPS-based mutation detection accurately identifies patients with mutations in genes leading to inherited cancer predisposition[41] and has been used successfully to identify the spectrum of variants in single populations of patients with colon, ovarian and uterine cancers[42-44]. Recently, studies have reported findings using multiplex panels in heterogeneous groups of *BRCA1/2* negative patients, either in randomly selected[45,46] or consecutive[47] patients from high risk genetics clinics or in all patient samples submitted to commercial testing laboratories[46,48]. Excluding monoallelic *MUTYH* carriers as the associated cancer risks are controversial[49,50], these studies of predominantly white individuals found that between 3.4-9.5% of *BRCA1/2* negative patients carried deleterious or likely deleterious variants in panel genes[45-48]. We found a deleterious or likely deleterious variant rate of 11% using a custom 22-gene panel in a well-characterized group of 278 early-onset breast cancer patients, including 66 African Americans, consistent with an increased likelihood of finding cancer susceptibility mutations in a younger, affected patient population. We found that 2.2% were heterozygous *MUTYH* carriers, similar to the LaDuca study rate of 1.7%[48] and the reported population carrier frequency of *MUTYH* mutations of 1.1% (range 0-2%)[51].

Our variant classification algorithm found a 19% VUS rate in the early-onset breast cancer patients using a pipeline integrating multiple data sources. Kurian et al. used only two *in silico* variant calling programs and population frequency data to analyze variants and reported a much higher 88% VUS rate. Our VUS rate is consistent with that in LaDuca et al. of 20% identified using Ambry's proprietary variant calling program, although lower than Tung et al of 42% using Myriad's variant calling method[46]. Given that VUSs cause confusion and anxiety for both patients and practitioners, incorporating various data sources to support calls and exploring novel variant classification methods will be increasingly necessary going forward.

In our study, we found that seven patients (2.5%) carried clinically reportable variants in *TP53*. Regarding the four individuals with *TP53* deleterious or likely deleterious variants, two had family histories meeting Chompret criteria, one was diagnosed at age 30 with bilateral breast cancer and one had a family history of late-onset sarcoma and multiple late-onset bilateral breast cancer cases; all were ascertained prior to 2007. No mutations were found in the genes associated with other well characterized cancer susceptibility syndromes, *PTEN*, *STK11*, and *CDH1*. Many of the patients in this study population were reviewed in a genetics conference at a tertiary care institution where there is high index of awareness for these phenotypes, and patients with known mutations in these genes were excluded from the present study. Their mutation rates may differ in unselected populations.

With regard to other high risk cancer susceptibility genes, one patient with a family history of melanoma was found to have a mutation in *CDKN2A*; excess breast cancer has been described in families with *CDKN2A* mutations[52]. One patient was found to have a likely deleterious variant in *MSH2* and one patient was a compound heterozygote for a *MUTYH* pathogenic mutation and a likely deleterious variant; the breast cancer risks associated with mutations in *MUTYH* and the mismatch repair genes such as *MSH2* is controversial[53,54]. It is possible that these mutations did not contribute to the development of breast cancer in these individuals. Further study of the breast cancer risks associated with these gene mutations is

needed. These data highlight the importance of determining the clinical management of individuals identified to have mutations by multiplex panel testing in genes not classically associated with the patient's phenotype or pedigree.

Regarding moderate risk breast cancer susceptibility genes, we found *ATM* mutations in 2.9% (n=8), *CHEK2* founder mutations (1100delC, I157T and c.444+1G>A) in 2.5% (n=7), and other *CHEK2* mutations in 1.4% (n=4) of patients. In addition, we found two patients with *MRE11A* mutations and single patients with mutations in *BARD1*, *BRIP1*, *NBN*, and *RAD50*, respectively. Interestingly, we did not identify any patients with *PALB2* or *RAD51C* mutations. It is possible that the ethnic diversity of our population (28% non-white) is responsible for the variability in mutation frequency between ours and other studies[55-61]. Our study demonstrates that mutations in individual moderate penetrance genes outside of *ATM* and *CHEK2* are likely very infrequent in patients with early-onset breast cancer.

There are a number of important limitations to our study. Our study design excluded individuals with a personal or family history of ovarian cancer and it is possible that such early-onset breast cancer patients will have a different spectrum of mutations. Our study also did not include genes recently proposed to contribute to breast cancer susceptibility such as *BLM*[62], *FANCC*[62], and *XRCC2*[63] or ovarian cancer susceptibility such as *RAD51D*[64], and mutations in these genes could be present in our study population. Massively parallel sequencing approaches have limitations in the identification of large genomic rearrangements and therefore these types of variants could still be present in our patient population. Finally, as the majority of patients in the study had a family history of breast cancer and were ascertained through two health systems and affiliated hospitals, our findings may not be generalizable to patients with early-onset breast cancer ascertained through population based studies.

Overall, our results suggest that at least 11% of *BRCA1/2* negative patients with early-onset breast cancer may have a causative mutation in high or moderate penetrance genes found on multiplex panel testing. A higher incidence of other malignancies may occur in early-

onset breast cancer patients with these mutations, and further study of these risks in larger populations could allow for more rational decision making regarding cancer screening and medical and/or surgical preventive treatments for these patients[5], for example prophylactic contralateral mastectomy at the time of a breast cancer diagnosis. In addition, it is now understood that the tumors in *BRCA1/2* carriers show increased sensitivity to PARP inhibitors and platinum agents due to synthetic lethality[65]. Given that many of the other cancer susceptibility genes studied here also play a role in double stranded DNA repair, it is possible that tumors of carriers of some of these other gene mutations may also show increased sensitivity to these agents[66].

Although our sample size was too limited to define the breast and non-breast cancer risks for family members of individuals with mutations in moderate penetrance genes, the Penn II and BOADICEA model prior probability scores were statistically significantly higher in deleterious or likely deleterious variant positive patients and this may reflect the stronger family histories of breast and/or other cancers in patients with deleterious mutations. Additional studies are needed to determine if true negative family members of those with mutations in the genes studied here can be counseled that they are at population risk for breast and other gene specific cancers, as is the case for *BRCA1/2*[5].

Our results highlight the critical need for large consortia to delineate the expected mutation rates, penetrance, and associated cancer risks for moderate risk genes found on cancer susceptibility genetic testing panels in well-defined clinical populations, keeping in mind the relatively lower penetrance of some of these mutations and the possibility for segregation of multiple risk alleles. In addition, consortia will be needed to pool data to study and develop clinical recommendations for patients carrying these mutations and their family members.

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Figure 1: Variants identified by multiplex panel testing of 278 patients with early onset breast cancer. Germline DNA from 278 *BRCA1/2* negative patients with early onset breast cancer (early-onset breast cancer) was isolated and subjected to massively parallel sequencing using a custom capture for the indicated genes in Bin A and Bin B. Sequencing data was analyzed with a custom bioinformatics pipeline and deleterious variants were called into classes (D = Deleterious, LD = Likely Deleterious, VUS = Variant of Uncertain Significance, LB = Likely Benign, and B = Benign). Inset: Proportion of patients self-reported as "White" or "Non-white" with deleterious or likely deleterious variants, VUSs only, or no reportable deleterious or likely deleterious variants or VUSs. The *MUTYH* heterozygous carriers included three patients heterozygous for a deleterious variant and three patients heterozygous for a VUS.

Figure 2: Representative family histories and sequencing data for three probands with identified mutations. A. Patient 5129, *TP53* c.451C>A, p.P151T found by massively parallel sequencing and confirmed by Sanger sequencing. B. Patient 1723, *TP53* c.733G>A, p.G245S found by massively parallel sequencing and confirmed by Sanger sequencing. C. Patient 5066, *ATM* c.8266A>T p.K2756X and *CHEK2* c.444+1G>A found by massively parallel sequencing and confirmed by Sanger sequencing in both the proband and her brother (arrows).

Conflict of interest statements

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Author(s): Kara N. Maxwell, Bradley Wubbenhorst, Kurt D'Andrea, Bradley Garman, Jessica M. Long, Jacquelyn Powers, Katherine Rathbun, Jill E. Stopfer, Jiajun Zhu, Angela R. Bradbury, Michael S. Simon , Angela DeMichele, Susan M. Domchek, and Katherine L. Nathanson (the "Author(s)")

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Cover Letter

Perelman School of Medicine
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September 24, 2014

James P. Evans
Editor in Chief
Genetics in Medicine

Re: Submission of Article

Dear Dr. Evans,

We are pleased to submit our Original Research Article “Prevalence of mutations in a panel of breast cancer susceptibility genes in *BRCA1/2* negative patients with early onset breast cancer” for consideration of publication in *Genetics in Medicine.*

In this manuscript, we present the first report of multiplex panel testing evaluating mutations in moderate and high penetrance breast cancer susceptibility genes in *BRCA1/2* negative patients with early onset breast cancer (diagnosed under age 40). As compared the paper published in GIM from Ambry Genetics, this cohort is well-characterized with accompanying clinical data. Using a massively parallel sequencing (MPS) panel of 22 inherited cancer susceptibility genes, we found deleterious variants in 11% of patients, higher than in prior studies as our population is particularly high risk. However, we highlight in our study that only seven patients (2.5% of the total population) have definitively actionable mutations given current clinical management guidelines. We report that 19% of patients have a Variant of Uncertain Significance (VUS), a figure similar to some but not all prior studies. Of particular interest to GIM readers, we provide details of our pipeline for variant classification, which is not included in other similar studies and provides important information about how our conclusions are reached for both clinicians and researchers in this field, and a model for future studies. Finally, we present associations of clinicopathological characteristics, comparing patients with and without mutations. We found a significantly higher rate of second primary malignancies in patients with deleterious variants (p= 0.02) and that models used to predict the probability of mutations in *BRCA1/2* also are predictive of mutations in other cancer susceptibility genes. In addition, the VUS rate is significantly higher in non-white patients compared to white patients (31% vs 15%, p=0.01). Our study provides important data for clinicians considering multiplex genetic panel testing for their high risk breast cancer patients and demonstrates the critical need for further research on the responsible clinical translation of this rapidly expanding technology. In summary, our study is the first report to our knowledge on the spectrum of mutations and variants in other cancer susceptibility genes in a well-defined cohort of *BRCA1/2* negative early onset breast cancer patients, a group for which many clinicians are currently using panel testing. Therefore, we feel our results have particular clinical relevance.

We have no competing interests to report.

Thank you for your time and consideration,

Kara N. Maxwell, MD, PhD
Katherine L. Nathanson, MD

Figure 1

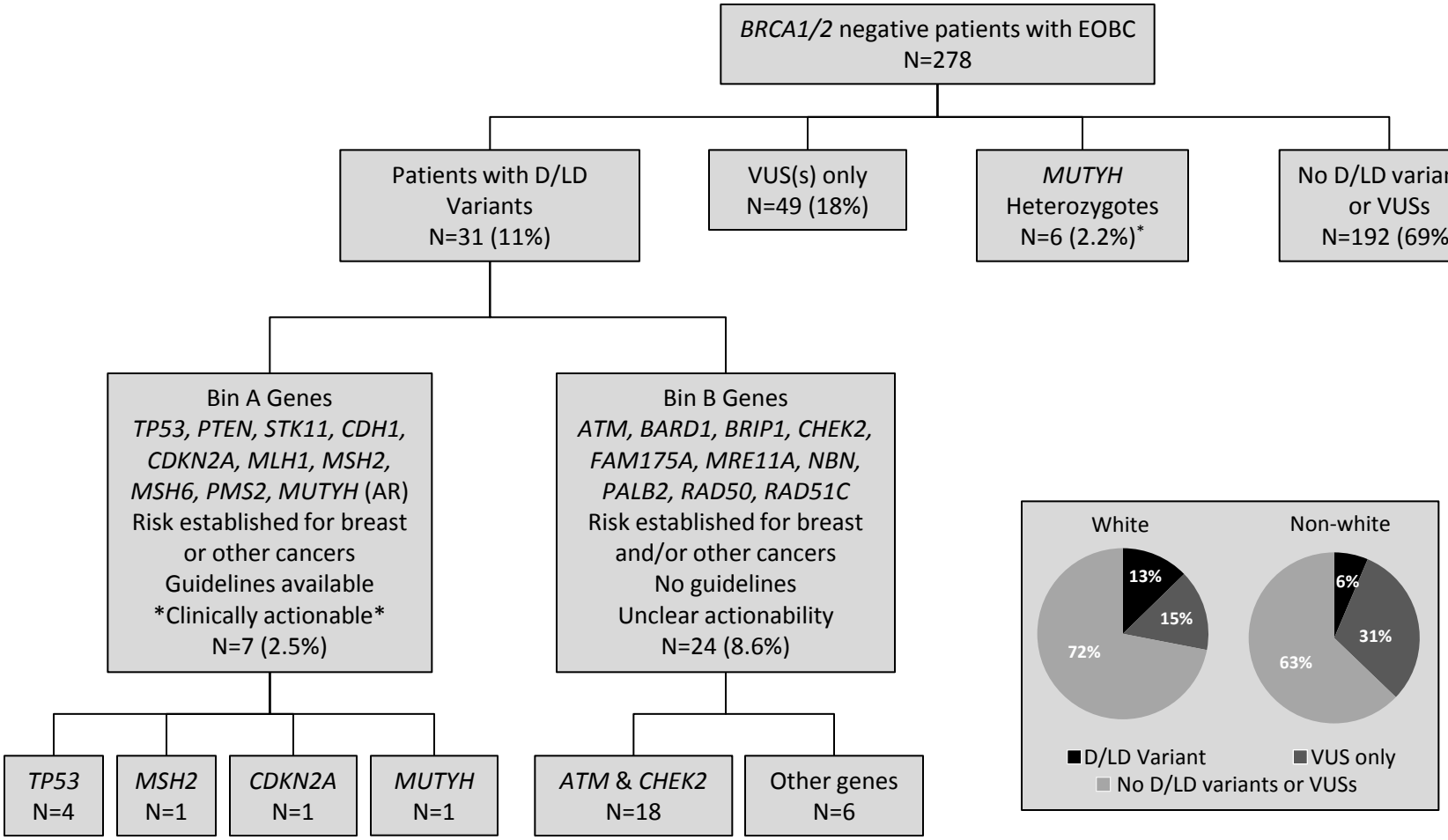

FIGURE 1

Figure 2

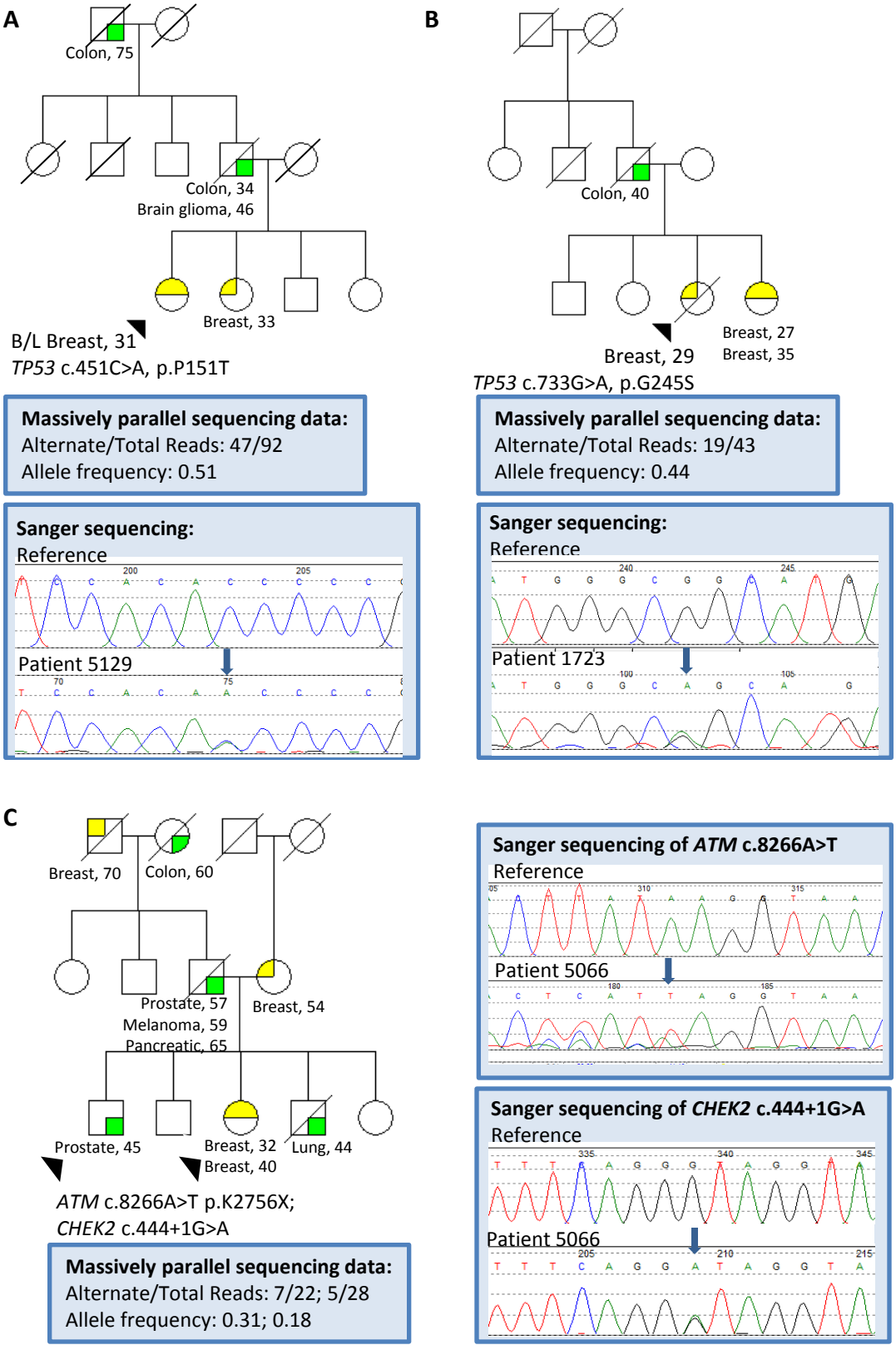

FIGURE 2

Table 1. Clinical and pathological characteristics of patients

Characteristic	Study population (n=278)	Mutation positive[a] (n=31)	VUS Positive[b] (n=55)	Mutation/ VUS negative[c] (n=192)	Mutation positive vs rest of population[d]
Clinical characteristics					***p-value***
Average age of onset of BC	34 (20-39)	34 (23-39)	34 (24-39)	34 (20-39)	NS
Self-reported race/ethnicity					
White/Caucasian	190 (69%)	24 (77%)	29 (53%)	136 (71%)	NS
African American/Black	66 (24%)	5 (16%)	19 (35%)	42 (22%)	NS
Other[e]	12 (4%)	0	5 (9%)	7 (4%)	NS
Not reported	10 (4%)	2 (7%)	2 (4%)	7 (4%)	NS
Ashkenazi Jewish	27 (10%)	6 (19%)	3 (5%)	18 (9%)	NS
Non-Jewish	234 (84%)	23 (74%)	51 (93%)	160 (83%)	NS
Personal cancer history					
Contralateral Breast Cancer	36 (13%)	6 (19%)	5 (9%)	25 (13%)	NS
2nd primary malignancy[f]	47 (17%)	6 (19%)	3 (5%)	12 (6%)	0.02
Family cancer history					
Breast cancer	188 (68%)	25 (81%)	35 (64%)	129 (67%)	NS
Breast cancer age<40	76 (27%)	9 (29%)	15 (27%)	52 (27%)	NS
Bilineal breast cancer	34 (12%)	7 (23%)	5 (9%)	22 (11%)	0.08
BRCA1/2 Prediction Models					
Penn II prior probability	21%	27%	20%	19%	0.04
BOADICEA	15%	29%	13%	14%	0.005

Pathological data	***N (%)***	***N (%)***	***N (%)***	***N (%)***	***p-value***
Ductal carcinoma in situ	23/278 (8%)	4/31 (13%)	7/55 (13%)	12/192 (6%)	NS
ER+ invasive BC	147/214 (69%)	19/22 (86%)	29/44 (66%)	99/148 (67%)	0.09

Her2+ invasive BC	49/175 (28%)	7/20 (35%)	10/30 (33%)	32/125 (26%)	NS
Stage I	67/208 (32%)	6/21 (29%)	14/41 (34%)	47/146 (32%)	NS
Stage IIA/B	96/208 (46%)	9/21 (43%)	16/41 (39%)	71/146 (49%)	NS
Stage IIIA/B/C	43/208 (21%)	6/21 (29%)	11/41 (27%)	26/146 (18%)	NS
Stage IV	2/208 (1.0%)	0/21	0/41	2/146 (1.4%)	n/a

[a]Including 30 patients with Deleterious and Likely Deleterious mutations and one *MUTYH* compound heterozygote

[b]Including patients with a VUS only or a single *MUTYH* variant

[c]Including patients with no Deleterious Variants, Likely Deleterious Variants or VUSs

[d]Comparisons were made using a two-tailed Fisher's exact test; except for comparison of age, Penn II scores, and BOADICEA scores which used a two-tailed, type 2 Student's t-test. Comparisons were also run for Mutation positive versus Mutation and VUS negative (excluding the VUS positive patients) and all p-values were consistent. NS = not significant.

[e]Other includes individuals of Asian descent (4), Hispanic/Latinos (6), and individuals reporting more than one race (2).

[f]Any malignancy, excluding non-melanoma skin cancer.

Table 2: Characteristics of patients with Deleterious or Likely Deleterious Variants

Variant(s)[a]	Proband Cancer	Race[b] and Family History[c]
TP53 c.451C>A, p.P151T (D)	Bilateral breast-31, ER-Her2+	Race: W; M: Breast age>40, lymphoma; P: Breast age<40, colon x2, brain
TP53 c.733G>A, p.G245S (D) *PALB2* c.94C>G, p.L32V **(**V)	Breast-29, Unk	Race: U; Sib: Bilateral breast age<40; M: Breast age>40; P: colon
TP53 c.374C>T, p.T125M (LD)	Bilateral breast-30, DCIS	Race: A; M: None; P: Unknown
TP53 c.1000G>C, p.G334R (LD)	Breast-37,Unk; Breast-65,Unk	Race: W/AJ; Sib: Colon; M: Breast age>40 x3, leukemia, lymphoma, kidney, sarcoma, melanoma; P: colon
CDKN2A c.104G>C, p.G35A (D) *MSH6* c.3203G>A, p.R1068Q **(**V)	Breast-38, Unk; Sarcoma-44	Race: W; M: Breast age>40, Melanoma; P: None
MSH2 del ex5 (LD)	Breast-39, ER+ Her2-	Race: W/AJ; M: None; P: thyroid, testicular
MUTYH c.1187G>A, p.G396D (D); *MUTYH* c.281G>A, p.R94Q **(**LD)	Colon-31; Breast-38, Unk; Breast-44, ER+Her2-	Race: W; M: Breast age<50, colon x3, uterine; P: gallbladder
ATM c.8856delTC (D)	Breast-39, ER+ Her2+	Race: W; M: None; P: pancreatic x2, bladder, unknown gastrointestinal
ATM c.2282delCT (D)	Breast-39, DCIS	Race: A; M: Breast age>40 x4; P: None
ATM c.6839delA (D)	Breast-34, DCIS	Race: W; M: Breast age<40 x6, breast age>40 x3, pancreatic, prostate, melanoma, brain; P: breast age>40 x3, pancreatic
ATM c.7271T>G, p.V2424G (D)	Breast-29, ER+ Her2-	Race: A; M: Breast age>40; P: rectal, lung, brain x2
ATM 8774G>T, p.G2925V (LD)	Breast-31, ER+ Her2-	Race: W; M: Breast age>40 x2, leukemia; P: None
ATM c.8155C>T, p.R2719C (LD)	Breast-38, ER+ Her2-	Race: A; M: Breast age>40; P: prostate
ATM c.8558C>G, p.T2853R (LD)	Breast-38, ER+ Her2-	Race: A; M: uterine; P: lung
ATM c.8266A>T, p.K2756X (D) *CHEK2* c.444+1G>A (D)	Breast-32, Unk; Breast-40, ER+ Her2-	Race: W; Sib: lung, prostate age 45; M: Breast age>40; P: prostate, melanoma, pancreatic, colon, breast age>40

CHEK2 c.1100delC (D)	Breast-32, ER+ Her2-	Race: W; M: melanoma, breast age>40 x2, colon x3, uterine; P: Breast age<40x2 & age>40x2, brain
CHEK2 c.1100delC (D)	Breast-38, ER+ Her2-	Race: W; M: lung, thyroid; P: lung x2
CHEK2 c.1100delC (D)	Melanoma-30; Breast-34, Unk	Race: W/AJ; M: Breast age<40 & age>40x3, prostate x4; P: None
CHEK2 c.1555C>T, p.R519X (D)	Breast-37, ER+	Race: W; M: Breast age>40, brain; P: None
CHEK2 c.444+1G>A (D)	Breast-32, ER-Her2+	Race: W; P: Breast age>40, prostate; M: Breast age >40x2, leukemia, pancreatic, unknown gastrointestinal
CHEK2 c.470T>C, p.I157T (D)	Breast-36, Unk; Breast-49, ER+ Her2-	Race: W; M: Breast age<40 & age>40 x2, lung; P: breast age>40
CHEK2 c.470T>C, p.I157T (D)	Breast-23, ER+ Her2+	Race: W/AJ; M: Breast age>40, testicular, colon; P: none
CHEK2 c.349A>G, p.R117G (D)	Wilms-2; Breast-33, ER+ Her2+	Race: W; M: None; P: prostate
CHEK2 c.1283C>T, p.S428F (D) *PMS2* c.944G>A, p.R315Q **(**V)	Breast-38, ER+Her2+	Race: W; M:adrenal, bladder, lung; P: None
CHEK2 c.499G>A, p.G167R (LD) *CHEK2* c.506T>C, p.F169S[d] **(**LD)	Breast-29, ER+ Her2-	Race: W/AJ; M: None; P: None; Sib (twin): breast age<40
BARD1 c.1652C>G, p.S551X (D)	Breast-35, Unk; Breast-39, TNBC	Race: W; M: None; P: Breast age>40
BRIP1 c.2992delTT (D)	Breast-35, ER+; Bladder-44	Race: W/AJ; M: Breast age>40, colon, liver; P: Breast age>40, Lung
MRE11A c.1378G>T, p.E460X **(**D)	Breast-36, ER+ Her2+	Race: W; M: Breast age<40, Breast age>40x2; P: lung
MRE11A c.1090C>T, p.R364X (D)	Breast-36, ER+	Race: W; M: None; P: Breast age>40x3, uterine
RAD50 c.1252delTT (D)	Breast-31, ER+ Her2-	Race: A; M: Breast, Bone; P: None
NBN c.664T>C, p.F222L (LD)	Breast-37, Unk; Leukemia-39	Race: W; M: Breast age>40x2, P: melanoma, prostate, bladder, lymphoma

[a]D: deleterious variant, LD: likely deleterious variant, V: variant of unknown significance (VUS). The method of variant classification is described in the Methods section. Data supporting call for missense variants is provided in Supplementary Table 1.

[b]W: White/Caucasian, A: African American, U: unknown; AJ: Ashkenazi Jewish descent

[c]M: Cancers found on the maternal side, P: Cancers found on the paternal side; Sib: cancers found in siblings

[d]The two *CHEK2* mutations were shown to be *in trans* by analysis of 250 sequencing reads in IGV.

Supplementary Table 1

Supplementary Table 1: Details used to classify variants									
Var #	dbSNP	VARIANTS LISTED HERE ARE ALL VARIANTS CALLED AS VUS, LD, OR D; ALSO ANY NOVEL VARIANTS ARE INCLUDED REGARDLESS OF CALL (note: for all nd = no data, n/a = not applicable)	FINAL CALL	STEP 1: Start as VUS if a) Novel (ESP, 1000g, dbSNP) OR b) LSDB PP>0.05		STEP 2: TALLY FEATURE D POINTS (0-5) and total possible (3-5)		FEATURE 1 - all variants: presence of mutation in functional domain = 1 D POINT	FEATURE 2 - all variants: CONSERVATION SCORE (1 D FOR SCORE>2 OUT OF 3)
				PRESENT IN DBSNP OR ESP OR 1000G?	LSDB PP or call (IARC or LOVD)	# D points	Total Possible of 5 features	Domain	Conserv. Score
1	0	ATM:NM_000051:exon25:c.T3601A:p.F1201I	LB	Novel	nd	0	3	none	1.34
2	0	ATM:NM_000051:exon26:c.G3778A:p.V1260M	VUS	Novel	nd	1	3	none	1.96
3	0	ATM:NM_000051:exon27:c.A4087G:p.T1363A	LB	Novel	nd	0	3	none	1.53
4	0	ATM:NM_000051:exon34:c.G5080A:p.A1694T	LB	Novel	nd	0	3	none	0.32
5	rs147187700	ATM:NM_000051:exon39:c.G5821C:p.V1941L	VUS	<0.1%	nd	3	5	none	2.82
6	rs28904921	ATM:NM_000051:exon49:c.T7271G:p.V2424G	D	<0.1%	Deleterious	4	5	FAT domain	2.39
7	rs147604227	ATM:NM_000051:exon50:c.C7313T:p.T2438I	VUS	<0.1%	nd	2	4	PIK-FAT	1.58
8	0	ATM:NM_000051:exon52:c.A7778G:p.Q2593R	LB	Novel	nd	0	3	none	1.39
9	rs138526014	ATM:NM_000051:exon56:c.C8155T:p.R2719C	LD	<0.1%	nd	4	5	PI3Kinase	2.27
10	0	ATM:NM_000051:exon58:c.C8558G:p.T2853R	LD	Novel	nd	4	4	PI3Kinase	2.74
11	0	ATM:NM_000051:exon60:c.G8774T:p.G2925V	LD	Novel	nd	4	4	PI3Kinase	2.69
12	0	ATM:NM_000051:exon62:c.C8938A:p.L2980I	LB	Novel	nd	0	3	none	0.87
13	rs80357024	BRCA1:NM_007294:exon10:c.T1233G:p.D411E	VUS	<0.1%	IARC PP 0.64 (de novo splice site)	1	4	none	-0.55
14	rs55650082	BRCA1:NM_007294:exon10:c.G1789A:p.E597K	VUS	<0.1%	IARC PP 0.02	3	5	none	2.02
15	0	BRCA1:NM_007294:exon10:c.G2468T:p.R823I	VUS	Novel	IARC PP 0.02	0	3	none	1.30
16	rs80356923	BRCA1:NM_007294:exon10:c.G3640A:p.E1214K	VUS	rsID only	IARC PP 0.02	4	4	none	2.56
17	0	BRCA2:NM_000059:exon3:c.T96A:p.F32L	VUS	Novel	IARC PP 0.29	5	5	none	2.50
18	rs28897701	BRCA2:NM_000059:exon3:c.G223C:p.A75P	VUS	<0.1%	IARC PP 0.02	3	5	none	2.84
19	rs80358726	BRCA2:NM_000059:exon6:c.C502A:p.P168T	VUS	rsID only	IARC PP 0.02	3	5	none	2.46
20	rs55854959	BRCA2:NM_000059:exon9:c.G742A:p.A248T	VUS	<0.1%	IARC PP 0.13	1	5	none	0.24
21	0	BRCA2:NM_000059:exon11:c.G6712A:p.D2238N	VUS	Novel	IARC PP 0.02	3	4	none	2.60
22	0	BRCA2:NM_000059:exon2:c.A62G:p.K21R	LB	Novel	IARC PP 0.03	0	4	none	0.05
23	0	BRCA2:NM_000059:exon10:c.C818T:p.S273L	LB	Novel	IARC PP 0.02	0	3	none	1.73
24	0	BRCA2:NM_000059:exon11:c.A2835C:p.K945N	LB	Novel	IARC PP 0.02	1	4	none	0.50
25	0	BRCA2:NM_000059:exon11:c.A3836G:p.N1279S	LB	Novel	IARC PP 0.02	0	3	none	0.32
26	0	BRCA2:NM_000059:exon11:c.G4813A:p.V1605I	LB	Novel	IARC PP 0.02	0	3	none	-0.41
27	0	BRCA2:NM_000059:exon11:c.C6463G:p.L2155V	LB	Novel	IARC PP 0.02	0	3	none	0.21
28	0	BRIP1:NM_032043:exon6:c.A587G:p.N196S	VUS	Novel	nd	1	3	Helicase	0.27
29	0	CDH1:NM_004360:exon3:c.G244A:p.V82M	LB	Novel	nd	0	3	Pro	1.26

Var #	dbSNP	VARIANTS LISTED HERE ARE ALL VARIANTS CALLED AS VUS, LD, OR D; ALSO ANY NOVEL VARIANTS ARE INCLUDED REGARDLESS OF CALL (note: for all nd = no data, n/a = not applicable)	FINAL CALL	STEP 1: Start as VUS if a) Novel (ESP, 1000g, dbSNP) OR b) LSDB PP>0.05		STEP 2: TALLY FEATURE D POINTS (0-5) and total possible (3-5)		FEATURE 1 - all variants: presence of mutation in functional domain = 1 D POINT	FEATURE 2 - all variants: CONSERVATION SCORE (1 D FOR SCORE>2 OUT OF 3)
				PRESENT IN DBSNP OR ESP OR 1000G?	LSDB PP or call (IARC or LOVD)	# D points	Total Possible of 5 features	Domain	Conserv. Score
30	0	CDKN2A:NM_000077:exon1:c.G104C:p.G35A	D	Novel	Pathogenic	4	5	ankryin	2.56
31	0	CHEK2:NM_007194:exon2:c.T163G:p.S55A	VUS	Novel	no LSDB	1	3	none	2.36
32	rs141568342	CHEK2:NM_007194:exon2:c.G190A:p.E64K	VUS	<0.1%	no LSDB	2	4	none	1.89
33	rs28909982	CHEK2:NM_007194:exon3:c.A349G:p.R117G	LD	<0.1%	no LSDB	5	5	FHA	2.16
34	rs17879961	CHEK2:NM_007194:exon4:c.T470C:p.I157T	D	<0.1%	Reported 32x	4	5	FHA	3.85
35	rs72552322	CHEK2:NM_007194:exon4:c.G499A:p.G167R	LD	<0.1%	no LSDB	5	5	FHA, phosphopeptide binding site	2.84
36	0	CHEK2:NM_007194:exon4:c.T506C:p.F169S	LD	Novel	no LSDB	3	3	FHA	2.12
37	0	CHEK2:NM_007194:exon11:c.A1169C:p.Y390S	LD	Novel	no LSDB	3	3	Kinase, T-loop activation segment	2.48
38	rs137853011	CHEK2:NM_007194:exon12:c.C1283T:p.S428F	D	<0.1%	no LSDB	5	5	Kinase	2.59
39	0	FAM175A:NM_139076:exon9:c.G892C:p.V298L	VUS	Novel	no LSDB	0	3	n/a	1.42
40	0	FAM175A:NM_139076:exon9:c.A1139G:p.D380G	VUS	Novel	no LSDB	0	3	n/a	1.20
41	rs201627097	FAM175A:NM_139076:exon9:c.G1082A:p.R361Q	VUS	rsID only	no LSDB	2	3	n/a	2.33
42	rs138705565	MLH1:NM_000249:exon1:c.G80A:p.R27Q	VUS	<0.1%	R27P Class 3	4	5	HATPase	2.88
43	0	MLH1:NM_000249:exon11:c.C945G:p.H315Q	VUS	Novel	nd	2	3	Transducer	2.21
44	rs63751049	MLH1:NM_000249:exon11:c.T977C:p.V326A	VUS	<0.1%	Class 1 PP <0.001; but hypomorphic	3	5	Transducer	2.47
45	rs147939838	MLH1:NM_000249:exon13:c.C1420G:p.R474G	VUS	<0.1%	R474W,Q Class 3	1	4	none	2.64
46	rs138584384	MLH1:NM_000249:exon19:c.C2173G:p.R725G	VUS	rsID only	R725H,C Class 3	3	5	none	2.17
47	0	MRE11A:NM_005590:exon5:c.G391A:p.D131N	VUS	<0.1%	nd	3	3	metalloprotease; DNA binding site	2.79
48	rs142996063	MRE11A:NM_005590:exon6:c.G529A:p.A177T	VUS	<0.1%	nd	3	3	metalloprotease	2.74

Var #	dbSNP	VARIANTS LISTED HERE ARE ALL VARIANTS CALLED AS VUS, LD, OR D; ALSO ANY NOVEL VARIANTS ARE INCLUDED REGARDLESS OF CALL (note: for all nd = no data, n/a = not applicable)	FINAL CALL	STEP 1: Start as VUS if a) Novel (ESP, 1000g, dbSNP) OR b) LSDB PP>0.05		STEP 2: TALLY FEATURE D POINTS (0-5) and total possible (3-5)		FEATURE 1 - all variants: presence of mutation in functional domain = 1 D POINT	FEATURE 2 - all variants: CONSERVATION SCORE (1 D FOR SCORE>2 OUT OF 3)
				PRESENT IN DBSNP OR ESP OR 1000G?	LSDB PP or call (IARC or LOVD)	# D points	Total Possible of 5 features	Domain	Conserv. Score
49	rs141711342	MSH2:NM_000251:exon1:c.T55C:p.F19L	VUS	<0.1%	nd	3	4	DNA Binding	2.32
50	0	MSH2:NM_000251:exon8:c.A1321C:p.T441P	VUS	Novel	Class 3	2	5	Core	0.54
51	rs41295288	MSH2:NM_000251:exon12:c.A1787G:p.N596S	VUS	<0.1%	Class 3	3	5	Core	2.42
52	0	MSH2:NM_000251:exon14:c.A2393G:p.N798S	VUS	Novel	nd	1	3	ATPase	1.50
53	rs41295296	MSH2:NM_000251:exon15:c.A2503C:p.N835H	VUS	rsID only	Class 3	4	5	HLH	2.14
54	0	MSH6:NM_000179:exon4:c.G1061T:p.G354V	VUS	Novel	nd	1	3	none	2.34
55	rs202219685	MSH6:NM_000179:exon4:c.A1231T:p.R411W	VUS	rsID only	nd	2	3	Mismatch binding	1.94
56	0	MSH6:NM_000179:exon4:c.G1870A:p.G624S	LB	Novel	nd	1	4	Connector	2.39
57	rs34938432	MSH6:NM_000179:exon4:c.G1932C:p.R644S	VUS	<0.1%	Class 3	1	5	Connector	-0.73
58	0	MSH6:NM_000179:exon5:c.G3203A:p.R1068Q	VUS	Novel	nd	0	3	Lever	0.24
59	rs191109849	MSH6:NM_000179:exon5:c.C3245T:p.P1082L	VUS	<0.1%	Class 3	2	5	ATPase	2.42
60	rs63750253	MSH6:NM_000179:exon5:c.G3284A:p.R1095H	VUS	rsID only	Class 3	3	5	ATPase	2.67
61	0	MSH6:NM_000179:exon6:c.G3478A:p.V1160I	VUS	<0.1%	nd	2	4	ATPase	1.80
62	rs36053993	MUTYH:NM_001128425:exon13:c.G1187A:p.G396D	D	<0.1%	Reported 509x	4	5	none	2.68
63	0	MUTYH:NM_012222:exon4:c.G355A:p.D119N	VUS	Novel	nd	2	4	endonuclease domain	1.89
64	0	MUTYH:NM_012222:exon3:c.G281A:p.R94Q	LD	Novel	nd	1	3	none	1.92
65	0	MUTYH:NM_012222:exon11:c.C962T:p.S321L	VUS	Novel	nd	0	3	none	0.16
66	rs144079536	MUTYH:NM_012222:exon13:c.C1249A:p.L417M	VUS	<0.1%	unknown	3	2	DNA glycosylase C	1.68
67	0	NBN:NM_002485:exon6:c.T664C:p.F222L	LD	Novel	no LSDB	4	4	none	2.50
68	rs182756889	NBN:NM_002485:exon5:c.C505T:p.R169C	VUS	<0.1%	nd	2	1	BRCT	-0.24
69	0	NBN:NM_002485:exon10:c.G1274A:p.R425K	LB	Novel	no LSDB	0	3	none	0.62

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				PRESENT IN DBSNP OR ESP OR 1000G?	LSDB PP or call (IARC or LOVD)	# D points	Total Possible of 5 features	Domain	Conserv. Score
70	0	PALB2:NM_024675:exon4:c.A1544G:p.K515R	VUS	Novel	inherited	2	4	ChAM-DNA binding	1.05
71	rs150390726	PALB2:NM_024675:exon1:c.C23T:p.P8L	VUS	<0.1%	inherited	3	4	coiled coil	0.65
72	rs151316635	PALB2:NM_024675:exon2:c.C94G:p.L32V	VUS	<0.1%	unknown	3	4	coiled coil	0.13
73	rs146176004	PMS2:NM_000535:exon2:c.G86C:p.G29A	VUS	<0.1%	nd	4	4	ATP binding	2.80
74	0	PMS2:NM_000535:exon3:c.C166G:p.L56V	VUS	Novel	nd	1	3	ATP binding	0.41
75	0	PMS2:NM_000535:exon6:c.A611G:p.N204S	VUS	Novel	nd	3	3	none	2.43
76	0	PMS2:NM_000535:exon6:c.G620A:p.G207E	VUS	<0.1%	nd	4	5	none	2.06
77	rs116314131	PMS2:NM_000535:exon9:c.G944A:p.R315Q	VUS	<0.1%	nd	2	3	MutS trans like	2.37
78	0	PMS2:NM_000535:exon10:c.G1096C:p.D366H	VUS	Novel	nd	0	3	none	1.38
79	0	RAD50:NM_005732:exon3:c.G260A:p.R87H	VUS	Reported	nd	2	3	ATPase	2.46
80	rs28903088	RAD50:NM_005732:exon5:c.G671A:p.R224H	VUS	<0.1%	nd	2	4	none	1.46
81	0	RAD50:NM_005732:exon11:c.G1679T:p.S560I	VUS	Novel	nd	0	3	none	0.79
82	0	RAD50:NM_005732:exon11:c.T1680G:p.S560R	VUS	Novel	nd	0	3	none	1.77
83	rs28903092	RAD50:NM_005732:exon13:c.G2177A:p.R726H	VUS	<0.1%	unknown	3	4	Zinc hook	2.65
84	rs138749920	RAD50:NM_005732:exon16:c.C2647T:p.R883C	VUS	<0.1%	nd	1	0	none	0.75
85	0	RAD50:NM_005732:exon21:c.G3358C:p.D1120H	VUS	Novel	nd	2	3	none	2.70
86	0	RAD50:NM_005732:exon25:c.A3824G:p.E1275G	VUS	<0.1%	nd	3	5	ATPase	2.50
87	0	RAD51C:NM_058216:exon4:c.C680T:p.P227L	VUS	Novel	nd	2	1	DNA recomb/repair;	1.92
88	0	STK11:NM_000455:exon8:c.C976A:p.P326T	LB	Novel	nd	0	3	none	-0.09

Var #	dbSNP	VARIANTS LISTED HERE ARE ALL VARIANTS CALLED AS VUS, LD, OR D; ALSO ANY NOVEL VARIANTS ARE INCLUDED REGARDLESS OF CALL (note: for all nd = no data, n/a = not applicable)	FINAL CALL	STEP 1: Start as VUS if a) Novel (ESP, 1000g, dbSNP) OR b) LSDB PP>0.05		STEP 2: TALLY FEATURE D POINTS (0-5) and total possible (3-5)		FEATURE 1 - all variants: presence of mutation in functional domain = 1 D POINT	FEATURE 2 - all variants: CONSERVATION SCORE (1 D FOR SCORE>2 OUT OF 3)
				PRESENT IN DBSNP OR ESP OR 1000G?	LSDB PP or call (IARC or LOVD)	# D points	Total Possible of 5 features	Domain	Conserv. Score
89	rs28934874	TP53:NM_000546:exon5:c.C451A:p.P151T	D	rsID only	22S/1G; unk SNP	5	5	DNA binding	2.72
90	rs28934575	TP53:NM_000546:exon7:c.G733A:p.G245S	D	<0.1%	454S/36G; unk SNP	5	5	DNA binding (essential residue)	2.38
91	0	TP53:NM_000546:exon4:c.C374T:p.T125M	LD	Novel	12S/0G; not SNP	4	5	DNA binding	2.44
92	0	TP53:NM_000546:exon10:c.G1000C:p.G334R	LD	Novel	0S/1G; unk SNP	5	5	tetramerization	n/a
93	0	TP53:NM_000546:exon5:c.C523T:p.R175C	VUS	Novel	28S/0G; not SNP	4	5	DNA binding (essential residue)	0.84
94	0	TP53:NM_000546:exon8:c.C868T:p.R290C	VUS	Novel	9S/0G; unk SNP	3	5	none	1.77
95	rs17881470	TP53:NM_000546:exon10:c.T1096G:p.S366A	VUS	rsID only	1S/2G; unk SNP	3	5	none; phosphorylation site	0.52
96	0	TP53:NM_000546:exon10:c.G1079T:p.G360V	LB	Novel	1S/0G; unk SNP	0	4	none	0.29

Supplementary Table 1: Details used to classify variants

Var #	FEATURE 3 - all variants: FUNCTIONAL SCORE (1 D FOR SCORE>4 OUT OF 5 OR 3-4 WITH CONDEL DEL CALL)			FEATURE 4 - Not all variants: Pathogenicity call in dbSNP, Clinvar or HGMD (1 D POINT IF CALLED deleteorious/pathogenic IN ANY DATABASE)					FEATURE 5 - Not all variants: PUBMED CALL (1 D POINT IF FUNCTIONALLY DELETERIOUS)	
	Func Score	GVGD (if available)	Condel Call	BIC	dbSNP	Clinvar	HGMD	Final curated database call (# supporting)	Pubmed	
1	2.58	Class C0	N	n/a	nd	nd	nd	nd	nd	
2	3.25	Class C15	D	n/a	nd	nd	nd	nd	nd	
3	1.13	Class C0	N	n/a	nd	nd	nd	nd	nd	
4	1.40	Class C0	N	n/a	nd	nd	nd	nd	nd	
5	3.71	Class C0	N	n/a	unflagged, nd	nd	DM A-T	D (1)	Defective ATM function in vitro in PMID:16014569; reported in A-T in 19431188	
6	3.58	Class C65	D	n/a	Pathogenic	Pathogenic	DM-A-T	D (3)	Reported in A-T patient in PMID: 8755918; in 3 brca cases in PMID: 17001622	
7	3.20	Class C0	N	n/a	unflagged, nd	nd	nd	nd	Reported in A-T patient, other is truncating mutation in PMID:10817650	
8	2.35	Class C0	N	n/a	nd	nd	nd	nd	nd	
9	4.43	Class C35	D	n/a	unflagged, nd	nd	R2719H DM BC	D (1)	R2719H reported PMID:19781682, comp pathogenicity PMID: 22529920	
10	4.66	Class C65	D	n/a	nd	nd	nd	nd	Fits criteria PMID:19781682	
11	4.74	Class C65	D	n/a	nd	nd	nd	nd	Fits criteria PMID:19781682	
12	2.25	Class C0	N	n/a	nd	nd	nd	nd	nd	
13	#VALUE!	Class C0	N	VUS	VUS	VUSx1	nd	VUS	nd	
14	4.06	Class C0	N	VUS	VUS	Benign x2	nd	VUS	Neutral per PMID:21990134	
15	3.48	Class C0	N	nd	nd	nd	nd	nd	nd	
16	4.03	Class C0	D	VUS	VUS	nd	nd	VUS	Prevents CK2A1 binding, alters phosphorylation (PMID: 23704879)	
17	4.37	Class C15	D	nd	nd	nd	DM-Breast cancer	D (1)	Necessary for PALB2 binding; PMID: 19609323; Reported in Japanese br ca family in PMID: 9609997	
18	3.82	Class C0	D	VUS	untested	benign	DM-Breast cancer	D (1), VUS (1)	VUS per PMID: 12845657; Not deleterious in PMID:18724707;	
19	4.34	Class C0	D	VUS	untested	nd	nd	VUS	Not deleterious in PMID: 17924331	
20	1.53	Class C0	N	VUS	untested	nd	DM-Breast cancer	D (1), VUS (1)	Reported as AA allele in PMID: 12491487; VUS in Nigerian patients in PMID: 15744044	
21	3.61	Class C0	D	VUS	nd	nd	nd	VUS	nd	
22	2.20	Class C0	N	nd	nd	VUSx1	nd	VUS	nd	
23	2.30	Class C0	D	nd	nd	nd	nd	nd	nd	
24	1.67	Class C0	D	nd	nd	VUSx1	nd	VUS	nd	
25	1.58	Class C0	N	nd	nd	nd	nd	nd	SNP per PMID: 12552570	
26	2.00	Class C0	N	nd	nd	nd	nd	nd	nd	
27	0.68	Class C0	N	nd	nd	nd	nd	nd	nd	
28	#VALUE!	n/a	N	n/a	nd	nd	nd	nd	nd	
29	1.74	n/a	N	n/a	nd	nd	nd	nd	nd	

Var #	FEATURE 3 - all variants: FUNCTIONAL SCORE (1 D FOR SCORE>4 OUT OF 5 OR 3-4 WITH CONDEL DEL CALL)			FEATURE 4 - Not all variants: Pathogenicity call in dbSNP, Clinvar or HGMD (1 D POINT IF CALLED deleteorious/pathogenic IN ANY DATABASE)					FEATURE 5 - Not all variants: PUBMED CALL (1 D POINT IF FUNCTIONALLY DELETERIOUS)	
	Func Score	GVGD (if available)	Condel Call	BIC	dbSNP	Clinvar	HGMD (DM = disease mutation)	Final curated database call (# supporting)	Pubmed	
30	#VALUE!	n/a	N	n/a	nd	nd	DM-Melanoma	D (1)	Pathogenic in PMID:8595405; 10070944; function defective in 19260062, 20340136, 21462282 but not 23190892	
31	3.15	Class C0	N	n/a	nd	nd	nd	nd	nd	
32	2.36	Class C0	N	n/a	unflagged, nd		DM-Prostate cancer	D (1)	Deleterious in functional assay in PMID:22419737 and 16835864	
33	3.93	Class C65	D	n/a	unflagged, nd	nd	DM-Breast cancer	D (1)	Deleterious in functional assay in PMID: 16982735; reported in 1 br ca family in PMID:12454775	
34	2.48	Class C0	n	n/a	pathogenic	pathogenic vs risk factor	DFP LFS	D (3)	OR BC 1.6 in PMID: 23713947 (meta-analysis 26,336 cases and 44,219 controls)	
35	4.95	Class C65	D	n/a	unflagged, nd	nd	DM-Prostate cancer	D (1)	Deleterious in DNA damage assay per PMID: 22419737; reported in 1 prostate cancer case and no controls in PMID: 12533788	
36	4.60	Class C35	D	n/a	nd	nd	nd	nd	nd	
37	4.92	Class C65	D	n/a	nd	nd	nd	nd	nd	
38	4.07	Class C15	D	n/a	other	risk factor	DFP Breast cancer	D (2)	AJ allele; 2-fold risk per PMID: 15649950	
39	2.86	n/a	N	n/a	nd	nd	nd	nd	nd	
40	2.03	n/a	N	n/a	nd	nd	nd	nd	nd	
41	3.85	n/a	D	n/a	unflagged, nd	nd	nd	nd	nd	
42	4.04	Class C35	D	n/a	Likely pathogenic	nd	R27P DM	D (2)	R27P report is 22290698	
43	3.65	Class C0	N	n/a	nd	nd	nd	nd	nd	
44	#VALUE!	Class C25	N	n/a	Unknown	VUS x1, benign x1	DM-HNPCC	Mixed	functionally defective; 30% MMR activity in vitro in PMID: 17510385 AND 11555625; reported in HNPCC family in PMID: 7585065	
45	#VALUE!	Class C15	N	n/a	unflagged, nd	nd	R474Q DM? HNPCC	D (1)	nd	
46	4.00	Class C45	D	n/a	unflagged, nd	nd	R755H DM? HNPCC	D (1)	Reported in HNPCC family in PMID: 18383312	
47	4.40	Class C15	D	n/a	nd	nd	nd	nd	nd	
48	4.74	Class C55	D	n/a	unflagged, nd	nd	nd	nd	nd	

Var #	FEATURE 3 - all variants: FUNCTIONAL SCORE (1 D FOR SCORE>4 OUT OF 5 OR 3-4 WITH CONDEL DEL CALL)			FEATURE 4 - Not all variants: Pathogenicity call in dbSNP, Clinvar or HGMD (1 D POINT IF CALLED deleteorious/pathogenic IN ANY DATABASE)					FEATURE 5 - Not all variants: PUBMED CALL (1 D POINT IF FUNCTIONALLY DELETERIOUS)	
	Func Score	GVGD (if available)	Condel Call	BIC	dbSNP	Clinvar	HGMD (DM = disease mutation)	Final curated database call (# supporting)	Pubmed	
49	4.54	Class C15	D	n/a	unflagged, nd	nd	nd	nd	nd	
50	2.10	Class C0	N	n/a	nd	VUS	DM-HNPCC	D (1), VUS (1)	Reported in Lynch patient in PMID: 1838331 but proposed neutral in 22290698	
51	2.75	Class C0	N	n/a	unknown	VUS x2	DM-HNPCC	D (1), VUS (2)	Proband w/mutation tumor MSH2 deficient; MSI-H in PMID: 16203774; but neutral in 22290698	
52	2.01	Class C0	N	n/a	nd	nd	nd	nd	nd	
53	2.66	Class C0	N	n/a	unflagged, nd	VUS	nd	VUS (1)	2 HNPCC probands in PMID: 19117025	
54	2.83	Class C0	N	n/a	nd	nd	nd	nd	nd	
55	4.83	Class C35	D	n/a	unflagged, nd	nd	nd	nd	nd	
56	3.61	Class C0	N	n/a	nd	nd	nd	nd	No impact bioinformatic analysis in PMID:23621914	
57	1.11	Class C0	N	n/a	unflagged, nd	VUS by expert panel	nd	VUS	No impact bioinformatic analysis in PMID:23621914	
58	1.30	Class C0	N	n/a	nd	nd	nd	nd	nd	
59	3.58	Class C0	N	n/a	unflagged, nd	VUS by expert panel	nd	VUS (1)	No impact bioinformatic analysis in PMID:23621914	
60	4.87	Class C25	D	n/a	unknown	VUS by expert panel	nd	VUS	WT activity in PMID: 24040339, 21120944	
61	4.05	Class C25	N	n/a	nd	nd	nd	nd	No impact bioinformatic analysis in PMID:23621914	
62	4.62	n/a	nd	n/a	pathogenic	DM-MAP		D	Het Y179C, G396D carriersBC OR of 1.9 PMID: 21952991); p.Y179C and p.G396D in70-80% of European MAP; 2/4 G396D hets in Ambry Colonext study had br ca	
63	2.45	n/a	N	n/a	nd	nd	nd	nd	Reduced activity in PMID 11756418	
64	3.90	n/a	N	n/a	nd	nd	nd	nd	Patient phenotype c/w biallelic MYH	
65	#VALUE!	n/a	N	n/a	nd	nd	nd	nd	nd	
66	3.63	n/a	N	n/a	unknown	nd	nd	VUS (1)	VUS in PMID: 21287799	
67	4.63	Class C15	D	n/a	nd	nd	DM-Melanoma	D (1)	a/w melanoma per PMID:17496786	
68	3.45	Class C0	D	n/a	unflagged, nd	nd	nd	nd	nd	
69	0.12	Class C0	N	n/a	nd	nd	nd	nd	nd	

Var #	FEATURE 3 - all variants: FUNCTIONAL SCORE (1 D FOR SCORE>4 OUT OF 5 OR 3-4 WITH CONDEL DEL CALL)			FEATURE 4 - Not all variants: Pathogenicity call in dbSNP, Clinvar or HGMD (1 D POINT IF CALLED deleteorious/pathogenic IN ANY DATABASE)					FEATURE 5 - Not all variants: PUBMED CALL (1 D POINT IF FUNCTIONALLY DELETERIOUS)	
	Func Score	GVGD (if available)	Condel Call	BIC	dbSNP	Clinvar	HGMD (DM = disease mutation)	Final curated database call (# supporting)	Pubmed	
70	2.31	n/a	N	n/a	nd	nd	nd	nd	Found in 1 WECARE participant PMID: 22241545	
71	2.10	n/a	N	n/a	unflagged, nd	nd	DM? BC	D (1)	Found in 1/139 AA brca cases in PMID: 21113654	
72	3.47	n/a	D	n/a	unflagged, nd	nd	nd	nd	Found in 1/747 kConFab in PMID: 23448497	
73	4.50	Class C55	D	n/a	unknown	VUS by one submitter	nd	VUS (2)	nd	
74	2.50	Class C0	N	n/a	nd	nd	nd	nd	nd	
75	4.51	Class C45	D	n/a	nd	nd	nd	nd	nd	
76	4.08	Class C0	D	n/a	nd	nd	DM-HNPCC	D (1)	Found in 1 Lynch patient in PMID: 19479271	
77	3.77	Class C0	N	n/a	unflagged, nd	nd	nd	nd	nd	
78	3.72	Class C0	N	n/a	nd	nd	nd	nd	nd	
79	3.91	Class C0	N	n/a	nd	nd	nd	nd	nd	
80	4.27	Class C0	D	n/a	unflagged, nd	nd	Breast/ovarian cancer?	D (1)	Found in 2% br/ov cases vs 1% controls in PMID: 14684699	
81	2.99	Class C0	N	n/a	nd	nd	nd	nd	nd	
82	3.00	Class C0	N	n/a	nd	nd	nd	nd	nd	
83	3.24	Class C0	N	n/a	unflagged, nd	nd	nd	nd	Found in 1/481 br cases in PMID: 16385572	
84	4.35	Class C0	D	n/a	unflagged, nd	nd	nd	nd	nd	
85	4.80	Class C65	D	n/a	nd	VUS by one submitter	nd	VUS (1)	nd	
86	3.97	Class C0	D	n/a	nd	nd	nd	nd	nd	
87	3.74	n/a	D	n/a	nd	nd	nd	nd	nd	
88	2.64	n/a	N	n/a	nd	nd	nd	nd	nd	

Var #	FEATURE 3 - all variants: FUNCTIONAL SCORE (1 D FOR SCORE>4 OUT OF 5 OR 3-4 WITH CONDEL DEL CALL)			FEATURE 4 - Not all variants: Pathogenicity call in dbSNP, Clinvar or HGMD (1 D POINT IF CALLED deleteorious/pathogenic IN ANY DATABASE)					FEATURE 5 - Not all variants: PUBMED CALL (1 D POINT IF FUNCTIONALLY DELETERIOUS)	
	Func Score	GVGD (if available)	Condel Call	BIC	dbSNP	Clinvar	HGMD (DM = disease mutation)	Final curated database call (# supporting)	Pubmed	
89	4.63	Class C35	D	n/a	unknown	nd	DM-LFS	D (1), VUS (1)	1 LFS family in PMID: 11479205; 14x in Cosmic; IARC: Non-func TA; Funcationally severe deficient in 17606709 and 21343334	
90	4.60	Class C55	D	n/a	pathogenic	pathogenic	DM-LFS	D (3)	1 LFS family in PMID:2259385 and 1 LFS in PMID:1978757; 301x in COSMIC IARC: Non-func TA	
91	4.48	Class C65	D	n/a	nd	nd	T125R DM-LFS	D (1)	nothing published; 12x in COSMIC; IARC: Non-func TA	
92	C	Class C65	D	n/a	nd	nd	DM-breast cancer	D (1)	1 LFS family in PMID: 23580068; IARC: Func TA	
93	4.67	Class C65	D	n/a	nd	nd	DM-ovarian cancer	D (1)	deleterious per MCK in PMID: 22006311 but retains in vitro WT function in PMID:9632751; 16x in COSMIC: IARC: Part-func TA	
94	4.25	Class C25	N	n/a	nd	nd	R290H & R290L DM-LFS	D (1)	40% WT function in vitro in PMID:17311302; 4x in COSMIC; IARC: Func TA	
95	2.13	Class C0	nd	n/a	Unknown	nd	DM-carcinoma	D (1)	S-366 phosphorylated by CHEK2 PMID:15659650; partial functional deficiency in 21343334; 2x in COSMIC; IARC Func TA	
96	1.84	Class C0	N	n/a	nd	nd	nd		nothing published; not in Cosmic; IARC: Func TA	

DOD BC123126 Request for Change in Specific Aim 2 8/4/2014

Original Aims of the Grant

Aim 1: To characterize the frequency and types of mutations in a panel of breast cancer susceptibility genes in women with early onset breast cancer

Aim 2: To characterize the frequency and types of mutations in a panel of breast cancer susceptibility genes in women with triple negative breast cancer

Aim 3: To develop methods of stratifying rare variants in breast cancer susceptibility genes by functionality to identify which variants should be considered for testing in future studies of clinical association with breast cancer and breast cancer outcomes

Proposed Change to Aim 2:

Aim 2: To characterize the frequency and types of mutations in a panel of breast cancer susceptibility genes in women with ***breast cancer and one or more other primary malignancies***

Data from Aim 1: In the first Aim of the grant, we have completed sequencing using a targeted panel of 28 genes in 278 women with early onset breast cancer. We have analyzed the variants identified in 22 genes which are found on commercially available breast cancer panels and have found that thirty-one patients (11%) have at least one deleterious (D) or likely deleterious (LD) variant. Fifty-four patients (19%) had at least one variant of uncertain significance (VUS) in a high or moderate penetrance gene, and six patients were heterozygous for a variant in *MUTYH*. Hormone receptor status was available on 22 of the 31 patients with D/LD variants. To our surprise, only three of the 22 patients with D/LD variants had ER- invasive breast cancer, one had triple negative breast cancer (*BARD1* S551X) and two had ER-Her2+ breast cancer (*TP53* P151T and *CHEK2* c.444+1A>G). There are 67 patients in our study so far with ER- breast cancer overall, and therefore 4.4% of ER- breast cancer patients were found to have D/LD variants. On the other hand, in comparison to D/LD variant negative patients, there was a statistically significant increase in the rate of second primary malignancies (excluding non-melanoma skin cancers, 19% vs 6%, p=0.02) in the D/LD variant positive patients. So far, we have 57 patients with early onset breast cancer and a second primary malignancy, and 12 patients (21%) have a D/LD variant.

Published Data: Data has been published in abstract form (American Society for Human Genetics in 2013, abstract 3263F, Bernier et al, University of Washington) on the frequency and types of mutations in 344 patients with triple negative breast cancer.

Rationale for changing patient population: Therefore, given that another research study is already near completion evaluating the frequency and types of mutations in triple negative breast cancer patients, the fact that we found a low rate of mutations in the ER- subgroup of our study but a fairly high rate of mutations in the multiple primary cancer subgroup, we would like to propose expanding this latter group for study. We have identified an additional 167 patients for which we have a DNA sample who have had breast cancer and a second primary malignancy (excluding non-melanoma skin cancer). In addition, we are working with our collaborator Dr. Angela Demichele to identify additional patients. We believe this is a unique data set where we have the opportunity to contribute valuable information to the field. In addition, we believe the multiple primary patient population will contribute more variants for study in Aim 3 of the grant.

Penn Medicine News

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This announcement is available online at
http://www.uphs.upenn.edu/news/News_Releases/2014/05/maxwell/

Large Panel Genetic Testing Produces More Questions than Answers in Breast Cancer

Researchers in Penn Medicine's Abramson Cancer Center Finds That Less than 3 Percent of Breast Cancer Mutations are Clinically Actionable

PHILADELPHIA — While large genetic testing panels promise to uncover clues about patients' DNA, a team of researchers from Penn Medicine's Abramson Cancer Center (ACC) has found that those powerful tests tend to produce more questions than they answer. In a study of 278 women with early onset breast cancer who did not have the BRCA genes, the researchers found that only 2.5 percent of the patients had inherited mutations that were actually clinically actionable. Experts don't yet know how to interpret most of the mutations discovered by the test—known as massively parallel gene sequencing.

Results of the study, led by author **Kara Maxwell, MD, PhD**, a fellow in the division of Hematology-Oncology in the Perelman School of Medicine at the University of Pennsylvania, will be presented during the annual meeting of the **American Society of Clinical Oncology (ASCO)** in Chicago in early June (**Abstract #1510**).

Large genetic testing panels sometimes reveal mutations in genes that are associated with an increased risk in developing cancer. BRCA 1 and BRCA 2 genes are prime examples, where women can opt for mastectomies and ovary removal surgery—which research shows slashes their risk of developing those cancers. However, there is not yet guidance for clinicians on how to care for patients who exhibit other types of mutations, such as CHEK2 and ATM. These are

known as variants of unknown significance (VUS).

“We’re in a time where the testing technology has outpaced what we know from a clinical standpoint. There’s going to be a lot of unknown variants that we’re going to have to deal with as more patients undergo large genetic testing panels,” said Maxwell. “It’s crucial that we figure out the right way to counsel women on these issues, because it can really provoke a lot of anxiety for a patient when you tell them, ‘We found a change in your DNA and we don’t know what it means.’”

The study, which includes senior authors **Susan Domchek, MD**, the Basser Professor in Oncology and director of the Basser Research Center for BRCA in Penn’s ACC, and **Katherine Nathanson, MD,** an associate professor in the division of Translational Medicine and Chief Oncogenomics Physician for the ACC, looked at 278 patients who had been diagnosed with breast cancer under the age of 40, were not carriers of the BRCA1 or BRCA2 mutations, and had no family history of ovarian cancer.

The researchers performed massively parallel gene sequencing to detect 22 known or proposed breast cancer susceptibility genes in each woman. Though the testing did reveal multiple variants of genes that are known to confer increased risk of breast cancer in patients who develop the disease young, only 2.5 percent of patients tested were found to have mutations that are actionable under current treatment guidelines, including TP53, CDKN2A, MSH2, and MUTYH.

In all, the sequencing revealed reportable variants in over 30 percent of the patients.

“Knowing there is a mutation may not help us any more than knowing that the person has a positive family history – which we already know,” Nathanson said. “We don’t know yet what to do with the information on an individual basis, and there certainly are no clinical standards.”

This field of research is especially important when dealing with families who appear to have genetic predisposition to breast or other cancers but don’t carry BRCA1/2 mutations, Maxwell said.

“We need to be very careful with how we use this data,” Maxwell said. “You could be taking someone who thinks they’re not at risk and making them at risk, or taking someone who is believed to be at risk and relieving them of that risk, but we don’t know enough yet to be confident in our assessments of these findings.”

The results will be presented at ASCO on Monday, June 2, 2014 during the “Next-Generation Sequencing Panels for Cancer Risk Assessment” Clinical Science Symposium from 8 a.m. to 9:30 a.m. in McCormick Place S100a.

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Penn Medicine consists of the Raymond and Ruth Perelman School of Medicine at the University of Pennsylvania (founded in 1765 as the nation's first medical school) and the University of Pennsylvania Health System, which together form a $4.3 billion enterprise.

The Perelman School of Medicine has been ranked among the top five medical schools in the United States for the past 17 years, according to U.S. News & World Report*'s survey of research-oriented medical schools. The School is consistently among the nation's top recipients of funding from the National Institutes of Health, with $392 million awarded in the 2013 fiscal year.*

The University of Pennsylvania Health System's patient care facilities include: The Hospital of the University of Pennsylvania -- recognized as one of the nation's top "Honor Roll" hospitals by U.S. News & World Report*; Penn Presbyterian Medical Center; Chester County Hospital; Penn Wissahickon Hospice; and Pennsylvania Hospital -- the nation's first hospital, founded in 1751. Additional affiliated inpatient care facilities and services throughout the Philadelphia region include Chestnut Hill Hospital and Good Shepherd Penn Partners, a partnership between Good Shepherd Rehabilitation Network and Penn Medicine.*

Penn Medicine is committed to improving lives and health through a variety of community-based programs and activities. In fiscal year 2013, Penn Medicine provided $814 million to benefit our community.